CHAPTER - III

RESULTS AND DISCUSSION

CHAPTER - III

RESULTS AND DISCUSSION

EXPERIMENT 1(a)

Growth rate and feed utilization in rats subjected to different degree of preweaning and/or postweaning food restriction.

As mentioned earlier, one of the major problems facing the world is undernutrition which is believed to affect body size and function. On the other hand, overnutrition is prevalent in some segments of the world's population.

The experiments described in this section were concerned with the effects of manipulating the plane of nutrition during the neonatal and postweaning periods on growth and food utilization.

The plane of nutrition during the neonatal period was sought to be manipulated by manipulating litter size to 4,8 or 12 pups per mother. The weaning weights of the three groups were mostly found to be in the range 45-50 g, 35-45 g, 25-35 g respectively. Animals outside this range were discarded. The animals in each category were divided into four groups, one of which was fed ad lib. During phase II (3-11 weeks of age) the other three groups were provided food in amounts representing 80, 66 and 50% of voluntary food intakes of the ad lib fed group. At the age of 11 weeks, they were all switched to an ad libitum feeding schedule (phase III).

The data on the body weight and food intake at the ends of phase II and III are presented in Tables 21-24.

It can be seen from Table 21 that in the male animals fed ad lib after weaning, the plane of nutrition during the neonatal period did not significantly affect body weights at the age of 11 weeks. Animals subjected to moderate undernutrition during the neonatal period achieved complete catch-up growth when fed ad lib during the postweaning period. This has been reported by other investigators (Knittle, 1972).

Failure to achieve complete catch-up has also been reported and this is probably associated with more severe degrees of undernutrition (Widdowson and McCance, 1960, 1963; Chow, 1964; Chow and Lee, 1964; Lee and Chow, 1965; Hsueh et al, 1967,1973, 1974; Blackwell et al, 1969; Rider and Simonson, 1973, 1974).

Postweaning deprivation resulted in growth retardation in all categories as expected, irrespective of the plane of neonatal nutrition. However, with food intakes of 80, 66 and 50% of ad lib, the body weights of male rats at 11 weeks of age were 85, 78 and 68 per cent of controls. The corresponding figures for body weight gains were 81, 74 and 64% respectively. These observations suggest that the animal responded to postweaning undernutrition with improved efficiency of tissue production as measured by weight gain per unit food intake.

Table 21 : Growth rate and efficiency of food utilization in male rats subjected to different degrees of postweaning food restriction in relation to preweaning nutritional status[1].

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± s.e.[b]				
		Body weight (g) at 11 weeks of age				
4	45 - 50	278 ± 7	246 ± 2**	219 ± 5**	195 ± 4**	235 ± 7
8	35 - 45	287 ± 6	236 ± 4**	218 ± 5**	190 ± 8**	233 ± 8
12	25 - 35	261 ± 8	217 ± 9**	208 ± 9**	185 ± 6**	218 ± 7
column mean[b]		275 ± 4	233 ± 4**	215 ± 4**	190 ± 3**	
		Body weights as % of control values				
4	45 - 50	100	89	79	70	
8	35 - 45	103	85	78	68	
12	25 - 35	94	78	75	67	
		Weight gain (g) during 3-11 weeks				
4	45 - 50	232 ± 6	197 ± 2**	173 ± 6**	148 ± 4**	188 ± 5
8	35 - 45	248 ± 7	198 ± 3**	177 ± 5**	151 ± 7**	194 ± 6
12	25 - 35	231 ± 7	178 ± 11**	177 ± 7**	157 ± 6**	186 ± 8
column mean		237 ± 3	191 ± 5**	176 ± 4***	152 ± 3***	

contd...

Table w 21 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Weight gain as % of control values				
4	45 - 50	100	85	76	64	
8	35 - 45	107	85	76	65	
12	25 - 35	100	77	76	68	
		Total food intake (g) 3-11 weeks				
4	45 - 50	988 ± 9	800	660	500	
8	35 - 45	1017 ± 20	800	660	500	
12	25 - 35	990 ± 21	800	660	500	
		Weight gain/g food intake				
4	45 - 50	0.235 ± 0.007	0.246 ± 0.003	0.262* ± 0.009	0.296*** ± 0.008	0.259 ± 00005
8	35 - 45	0.244 ± 0.005	0.248 ± 0.004	0.268* ± 0.008	0.302** ± 0.014	0.265 ± 0.006
12	95 - 35	0.233 ± 0.009	0.223 ± 0.014	0.268* ± 0.009	0.314*** ± 0.012	0.258 ± 0.009
column mean		0.237 ± 0.007	0.239 ± 0.007	0.266 ± 0.004	0.304 ± 0.010	

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Table 21 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intake (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Weight gain per g food intake (% control)				
4	45 - 50	100	105	111	126	
8	35 - 45	104	106	114	129	
12	25 - 35	99	95	115	134	

1. Preweaning nutritional status manipulated as shown in columns 1,2 and postweaning upto 11 weeks of age, as shown in columns 3,4,5 and 6.

a. Values based on 6-7 observations. For tests of significance all groups compared with the best nourished group (litter size, 4, weaning weight, 45-50 g, and fed ad libitum after weaning). One, two and three asterisks respectively indicate significance at p values less than 0.05, 0.01 and 0.001.

b. Row means compared with values for litter size 4. Column means compared with groups fed ad lib after weaning.

The pattern for females was essentially similar (Table 22) except that the response to food restriction as judged by weight gain as per cent of control was much less marked, as borne out by the following data :

Litter size	Weaning weight (g)	Postweaning diets as % ad lib intakes (3-11 weeks)							
		100		80		66		50	
		M	F	M	F	M	F	M	F
		weight gain as % controls (3-11 weeks)							
4	45-50	100	100	85	94	76	92	64	80
8	35-45	107	102	85	98	76	74	65	65
12	25-35	100	103	77	101	76	75	68	65

As is evident from the above, 80% food restriction did not have any effect on weight gain the females. The differences between males and females, however, were not seen in animals subjected to both neonatal and postweaning nutritional stress.

A comparison of the efficiency of food utilization for tissue production for males and females showed a pattern corresponding to the pattern seen for weight gains, as is evident from the following data :

Litter size	Weaning weight (g)	Postweaning diets as % ad lib intakes (3-11 weeks)							
		100		80		66		50	
		M	F	M	F	M	F	M	F
4	45-50	0.235	0.244	0.246	0.287	0.262	0.341	0.296	0.392
8	35-45	0.244	0.251	0.248	0.299	0.268	0.279	0.302	0.318
12	25-35	0.233	0.251	0.223	0.307	0.268	0.293	0.314	0.315

Table 23 shows that on rehabilitation male rats which had weaning weights greater than 35 g and were mildly (80% of ad lib) or moderately (66% of ad lib) undernourished postweaning caught up with the controls at the age of 16 weeks. It is interesting to note that this catch-up on rehabilitation is made not by significantly greater food intake compared to controls as one would expect, but by improved tissue production efficiency. The average food intake per week of the ad lib control group during phase III was more than during phase II in both males and females. However, as compared to males, the percentage increase in food intake of females was more (See Tables 23, 24). An increase in efficiency of food utilization in the face of food shortages is understandable, but what is remarkable is that the efficiency improved still further during the period of rehabilitation (See Tables 23,24) in the case of those animals which were mildly undernourished earlier and had achieved a moderate improvement in their tissue production efficiency.

Table 22 : Growth and efficiency of food utilization in female rats subjected to different degrees of postweaning food restriction in rel tion to preweaning nutritional status[1].

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± s.e.[a]				
		Body weight (g) at 11 weeks of age				
4	45 - 50	200 ± 5	191 ± 4	188 ± 3	168 ± 6**	187 ± 4
8	35 - 45	195 ± 4	188 ± 5	152 ± 5***	136 ± 4***	179 ± 4
12	25 - 35	191 ± 5	181 ± 5**	149 ± 7***	126 ± 4***	160 ± 5***
Column mean[b]		195 ± 3	186 ± 3**	160 ± 6***	140 ± 4***	
		Body weights as % of control values				
4	45 - 50	100	96	94	84	
8	35 - 45	98	94	76	68	
12	25 - 35	96	91	75	63	
		Weight gain (g) during 3-11 weeks of age				
4	45 - 50	152 ± 4	143 ± 4	140 ± 9	122 ± 6***	139 ± 6
8	35 - 45	156 ± 5	149 ± 6	113 ± 7***	99 ± 3***	130 ± 5
12	25 - 35	156 ± 6	153 ± 4	115 ± 7***	98 ± 4***	131 ± 5
Column mean		155 ± 3	149 ± 3	122 ± 5***	105 ± 3***	

contd...

Table 22 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Weight gain as % of control values				
4	45 - 50	100	94	92	80	
8	35 - 45	102	98	74	65	
12	25 - 35	103	101	75	65	
		Total food intake (g) 3-11 weeks of age				
4	45 - 50	624 ± 6	500	400	315	
8	35 - 45	621 ± 9	500	400	315	
12	25 - 35	623 ± 10	500	400	315	
		Weight gain (g) per g food intake				
4	45 - 50	0.244 ± 0.009	0.287 ± 0.008**	0.341** ± 0.022	0.392*** ± 0.019	0.315 ± 0.014
8	35 - 45	0.251 ± 0.008	0.299** ± 0.012	0.279* ± 0.013	0.318*** ± 0.010	0.284 ± 0.007
12	25 - 35	0.251 ± 0.008	0.307*** ± 0.008	0.293* ± 0.015	0.315*** ± 0.013	0.289 ± 0.007
Column mean		0.249 ± 0.005	0.299 ± 0.005	0.297 ± 0.012	0.338*** ± 0.010	

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Table 22 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Weight gain (g) per g food intake (% control)				
4	45 - 50	100	118	140	161	
8	35 - 45	103	123	122	130	
12	25 - 35	103	126	120	129	

1. Preweaning nutritional status manipulated as shown in columns 1,2 and postweaning, upto 11 weeks of age as shown in columns 3,4,5,6.

a. Values based on 6-7 observations.
For tests of significance all groups compared with the best nourished group (litter size 4, weaning weight, 45-50 g, and fed ad libitum after weaning). One, two and three asterisks respectively indicate significance at p values less than 0.05, 0.01 and 0.001.

b. Row means compared with values for litter size 4. column means compared with group fed adlib after weaning.

Table 23 : Growth rate and efficiency of food utilization in response to dietary rehabilitation in male rats subjected to different degrees of preweaning and/or postweaning food restriction.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± s.e.[a]				
		Body weight (g) at 16 weeks of age				
4	45 - 50	375 ± 12	384 ± 11	356 ± 11	316 ± 11**	358 ± 7
8	35 - 45	395 ± 8	372 ± 13	349 ± 8	316 ± 7**	358 ± 7
12	25 - 35	362 ± 14	340 ± 8*	333 ± 8*	311 ± 4***	338 ± 5
Column mean[b]		376 ± 7	365 ± 7	347 ± 5**	314 ± 3***	
		Body weight as % control values				
4	45 - 50	100	102	95	84	
8	35 - 45	105	100	93	84	
12	25 - 35	97	91	89	83	
		Weight gain (g) during 11-16 weeks of age				
4	45 - 50	100 ± 13	138 ± 10*	137 ± 10*	121 ± 6	124 ± 5
8	35 - 45	109 ± 8	137 ± 10*	131 ± 9	122 ± 11	126 ± 5
12	25- 35	117 ± 14	125 ± 16	111 ± 11	124 ± 5	119 ± 5
Column mean		109 ± 6	133 ± 6*	126 ± 6	123 ± 4	

contd...

Table 23 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intake (3-11 weeks)				Raw mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Weight gain as % control values				
4	45 - 50	100	138	137	121	
8	35 - 45	109	137	131	122	
12	25 - 35	117	125	111	124	
		Total food intake (g): 11-16 weeks of age				
4	45- 50	757 ± 16	775 ± 20	787 ± 11	798 ± 9*	779 ± 7
8	35 - 45	767 ± 7	790 ± 11	773 ± 9	735 ± 15	766 ± 6
12	25 - 35	757 ± 8	735 ± 15	748 ± 16	750 ± 6	748 ± 5***
Column mean		760 ± 6	767 ± 10	769 ± 7	761 ± 9	
		Weight gain (g) per g food intake				
4	45 - 50	0.144 ± 0.005	0.178** ± 0.008	0.174* ± 0.010	0.151* ± 0.006	0.161 ± 0.005
8	35 - 45	0.142 ± 0.010	0.173* ± 0.011	0.168 ± 0.010	0.171* ± 0.012	0.163 ± 0.005
12	25 - 35	0.154 ± 0.017	0.166 ± 0.016	0.147 ± 0.012	0.168* ± 0.007	0.159 ± 0.006
Column mean		0.147 ± 0.007	0.172* ± 0.006	0.163 ± 0.006	0.163 ±0.005	

contd...

Table 23 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intake (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Weight gain (g) per g food intake (% control)				
4	45 - 50	100	124	121	105	
8	35 - 45	99	120	117	119	
12	25 - 35	107	115	102	117	
		Food intake (g) per day per 100 g body weight at 16 weeks of age				
4	45 - 50	6.1 ± 0.3	5.8 ± 0.3	6.2 ± 0.3	7.1 ± 0.2*	6.3 ± 0.2
8	35 - 45	5.7 ± 0.2	5.8 ± 0.2	5.7 ± 0.4	6.8 ± 0.1*	6.0 ± 0.1
12	25 - 35	6.2 ± 0.2	6.1 ± 0.3	6.2 ± 0.2	6.7 ± 0.1	6.3 ± 0.1
Column mean		6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	6.9 ± 0.1***	
		Food intake per day per sq.cm. body surface area at 16 weeks of age[c]				
4	45 - 50	0.058	0.055	0.057	0.065	0.059
8	35 - 45	0.056	0.053	0.053	0.059	0.055
12	25 - 35	0.054	0.057	0.055	0.060	0.057
Column mean		0.056	0.055	0.055	0.061	

contd...

Table 23 : contd.

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1. Preweaning nutritional status manipulated as shown in Columns 1,2 and postweaning upto 11 weeks of age as shown in columns 3,4,5 and 6.

a. Values based on 6-7 observations.
For tests of significance all groups compared with the best nourished group (litter size 4, weaning weight, 45-50 g and fed ad libitum after weaning). One,two and three asterisks respectively indicate significance at p values less than 0.05, 0.01 and 0.001.

b. M Row means compared with values for litter size 4. Columns means compared with group fed ad lib after weaning.

c. Values obtained for means of each group.

Table 24 : Growth rate and efficiency of food utilization in response to dietary rehabilitation, in female rats subjected to different degrees of preweaning and/or postweaning food restriction[1].

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± s.e.[a]				
		Body weight (g) at 16 weeks of age				
4	45 - 50	243 ± 5	230 ± 7	223 ± 9	210 ± 3***	227 ± 4
8	35 - 45	236 ± 6	224 ± 9	203 ± 2***	198 ± 2***	216 ± 4
12	25 - 35	227 ± 6	210 ± 4***	197 ± 3***	190 ± 3***	203 ± 3***
Column mean[b]		233 ± 4	219 ± 3	208 ± 4***	206 ± 4***	
		Body weight as % of control values				
4	45 - 50	100	95	92	86	
8	35 - 45	97	93	84	82	
12	25 - 35	89	86	81	78	
		Weight gain (g) during 11-16 weeks of age				
4	45 - 50	43 ± 5	39 ± 7	35 ± 5	42 ± 4	39 ± 3
8	35 - 45	40 ± 5	36 ± 6	51 ± 5	62 ± 4*	47 ± 3
12	25 - 35	31 ± 6	28 ± 4	51 ± 10	63 ± 6*	43 ± 4
Column mean		37 ± 3	33 ± 3	46 ± 4	57 ± 4	

contd...

Table 24 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Weight gain as % of control values				
4	45 - 50	100	91	81	100	
8	35 - 45	93	81	119	144	
12	25 - 35	72	65	119	144	
		Total food intake (g) 11-16 weeks of age				
4	45 - 50	563 ± 18	592 ± 25	525 ± 24	512 ± 11*	551 ± 11
8	35 - 45	577 ± 13	552 ± 16	603 ± 23	603 ± 6	584 ± 9*
12	25 - 35	543 ± 12	566 ± 7	561 ± 23	596 ± 5	567 ± 8
Column mean		569 ± 8	546 ± 10	575 ± 16	576 ± 9	
		Weight gain (g) per g food intake				
4	45 - 50	0.072 ± 0.007	0.064 ± 0.011	0.066 ± 0.008	0.082 ± 0.006	0.071 ± 0.004
8	35 - 45	0.069 ± 0.007	0.065 ± 0.009	0.081 ± 0.007	0.103*** ± 0.007	0.079 ± 0.009
12	25 - 35	0.057 ± 0.007	0.054 ± 0.007	0.080 ± 0.010	0.105* ± 0.010	0.074 ± 0.005
Column mean		0.064 ± 0.004	0.060 ± 0.005	0.076 ± 0.005	0.099*** ± 0.005	

contd...

Table 24 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Weight gain per g food intake (% control)				
4	45 - 50	100	89	92	114	
8	35 - 45	96	90	113	143	
12	25 - 35	79	75	111	146	
		Food intake (g) per day per 100 g body weight at 16 weeks of age				
4	45 - 50	6.8 ± 0.2	6.8 ± 0.4	7.2 ± 0.5	8.1 ± 0.2***	7.1 ± 0.2
8	35 - 45	7.1 ± 0.3	6.9 ± 0.3	8 .4 ± 0.3***	8.5 ± 0.2***	7.7 ± 0.2*
12	25 - 35	7.0 ± 0.3	7.0 ± 0.2	8.1 ± 0.2***	8.2 ± 0.2***	7.7 ± 0.1**
Column mean		7.1 ± 0.2	6.9 ± 0.1	7.9 ± 0.2**	8.2 ± 0.1***	
		Food intake per day per sq. cm body surface area at 16 weeks of age[c]				
4	45 - 50	0.051	0.056	0.057	0.063	0.057
8	35 - 45	0.059	0.057	0.065	0.066	0.062
12	25 - 35	0.058	0.056	0.062	0.064	0.060
Column mean		0.059	0.056	0.061	0.064	

contd...

Table 24 : contd.

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1. Preweaning nutritional status manipulated as shown in columns 1,2 and postweaning upto 11 weeks of age as shown in columns 3,4,5 and 6.

a. Values based on 6-7 observations.
For tests of significance all groups compared with the best nourished group (litter size 4, weaning weight, 45-50 g and fed ad libitum after weaning). One, two and three asterisks respectively indicate significance at p values less than 0.05, 0.01 and 0.001.

b. Row means compared with values for litter size 4. Column means compared with groups fed ad lib after weaning.

c. Values obtained for means of each group.

This suggests that the metabolic patterns acquired during periods of food restriction may persist evenwhen food is available in plenty. This is consistent with the observations with regard to protein turnover to be documented in a subsequent section. Onthe other hand, these animals which had achieved a high efficiency of tissue production as a consequence of severe undernutrition did not show a similar improvement when rehabilitated, although they continued to do better than the controls (See Tables 22,24). With theexception of the group neonatally well nourished but severely(50% ad lib) undernourished after weaning, the undernourished animals.did not consume more food than the controls in absolute terms, but ate more in relation to either body weight or bodysurface.

Table24 shows that the females showing significant growth retardation at 11 weeks failed to catch-up even after 5 weeks of ad lib diet although they tended to achieve greater gains in weight gain the controls, the differences being significant in the case of the groups subjected to greater nutritional stressbefore rehabilitation. These were also the groups which compared favourably with the controls with regard to the efficiency of utilization of food for tissue production.

While in the early postweaning period (3-11 weeks of age), feed efficiency in males and females was of the same order, after 11 weeks it was less in females, a fact consistent with the differences in growth patterns.

These results are consistent with the pattern derived by Nolen (1972). In order to study the effect of varying degrees of postweaning food restriction on life, span, he fed male and female rats 60 or 80% ad lib diets. An analysis of the data presented by him shows weight gains of 69 and 83% of controls respectively in males and 75 and 91% a in females at 12 weeks of restriction. Essentially similar observations have been made in this laboratory both during undernutrition (Rajalakshmi and Ramakrishnan, 1969(a)) and during rehabilitation (Pillai, 1970; Parmeshwaran, 1974; Rajalakshmi and Ramakrishnan,1969(a)).

The superior adaptation of females as compared to males to undernutrition after weaning is also seen in the data reported by Nolen (1972).

It would be of interest to find out whether similar sex differences exist with regard to the response to rehabilitation. That the effect of neonatal manipulation through litter size persisting after postweaning rehabilitation was considerably greater in males than in females has been mentioned by Widdowson and Coworkers (Widdowson and McCance,1960; Widdowson and Kennedy, 1962; Widdowson, 1967). Also, in mice, neonatal deprivation induced by feeding mothers wheat gluten diets instead of casein during gestation and lactation had a more pronounced effect on the body weight of male progeny after 7 weeks of rehabilitation postweaning (Davies et al, 1973). But, Blackwell and coworkers (1969) working in the same laboratory have reported no differences in subsequent performance of male and female progeny of mothers fed 50% of ad lib intakes during gestation and/or lactation. Also, analysis of Nolen's data (1972) suggests no difference between males and females after rehabilitation. In the present studies only females subjected to neonatal stress performed better wk than males during rehabilitation. However, rehabilitation of females after postweaning protein deprivation has also been reported to be faster than the males (Lee, 1976; Radhakrishnan, 1976).

None of the above workers discuss possible reasons for the sex differences observed. A clue is perhaps provided by Naismith's studies on nitrogen metabolism in pregnant rats

(Naismith and Fears,1971,1972; Naismith, 1973). He found that pregnant rats were able to retain additional nitrogen when compared to pair fed non-pregnant controls. The livers of these animals produced less urea and a greater proportion of the protein ingested was presumably available for tissue production. Progesterone was found responsible for this anabolic effect, exerted indirectly through the intermediary functioning of the adrenal cortex. The normal blood level of progesterone in pregnant females is 0.28 µg/100 ml plasma compared to 0.1 - 0.3 for non-pregnant females. Males have a blood progesterone level of 0.03 µg % (Kutsky,1973). The higher levels in females as compared to males suggest an anabolic effect in deprived females.

Table 25 presents the body weights of males at later stages of life till the age of 52 weeks. Although a very consistent picture does not emerge, it can be said that the effect of moderate neonatal deprivation (litter size 12, weaning weights 25-35 g) and/or moderate (66% ad lib) or severe (50% ad lib) early postweaning deprivation was not completely overcome even after long periods of rehabilitation. That the effect of early postweaning food restriction to 60% ad lib is not completely overcome even after 2 years of rehabilitation has also been reported by Nolen (1972). However, Lee (1976) found complete catch-up in males at the age of 49 weeks and in females at the age of 21 weeks when rehabilitated after protein deficiency during 6-14 weeks of age.

Table 25 : Adult weights of rats subjected to different degrees of preweaning and/or postweaning food restriction and subsequent rehabilitation[1].

Litter size	Weaning weight (g)	Postweaning diet as % ad lib intakes (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± s.e.[a]				
		Body weight at 32 weeks of age				
4	45 - 50	559 ± 7	563 ± 6	537 ± 3*	506 ± 3***	541 ± 8
8	35 - 45	565 ± 6	547 ± 3	529 ± 4**	491 ± 3**	533 ± 7
12	25 - 35	548 ± 6	550 ± 6	518 ± 2***	485 ± 3***	525 ± 6
Column mean		557 ± 4	553 ± 2	528 ± 5**	494 ± 3***	
		Body weight at 52 weeks of age				
4	45 - 50	661 ± 17	657 ± 11	653 ± 4	656 ± 4	657 ± 5
8	35 - 45	650 ± 4	668 ± 3	662 ± 2	666 ± 3	662 ± 4
12	25 - 35	638 ± 4*	641 ± 4*	633 ± 3**	643 ± 5	639 ± 5
Column mean		650 ± 6	655 ± 5	649 ± 5	655 ± 4	

1. Preweaning nutritional status manipulated as shown in columns 1,2 and postweaning upto 11 weeks of age, as shown in columns 3,4,5 and 6.

a. Values based on 6-7 observations.
For tests of significance, all groups compared with the best nourished group (litter size 4, weaning weight, 45-50 g and fed ad libitum after weaning). One, two and three asterisks respectively indicate significance at p values less than 0.05, 0.01 and 0.001.

b. Row means compared with values for litter size 4, column means compared with group fed ad lib after weaning.

Experiment - 1(b)

Behavioral responses in the open field and activity wheel of male rats subjected to different degrees of preweaning and/or postweaning food restriction.

The association of malnutrition with apathy and inertia (Platt, 1961) has led to several studies on the effects of nutritional stress on 'activity' in experimental animals, especially rats. The activity may be voluntary or 'spontaneous' as in the case of locomotion in an open field (Whimbley and Denenberg, 1967) or the animal may be placed in such a situation that it is more or less obliged to exert itself to maintain equilibrium as in the case of the activity wheel. Additional indicators of activity are rearing, head raising, pivoting and urination and defecation in the test situation.

Protein deficiency in the postweaning period has been reported to decrease spontaneous activity (Rajalakshmi and Ramakrishnan, 1969(a). Persisting deficits in activity in adult life have been found in animals subjected to malnutrition during the fetal, neonatal and/or postweaning periods, even when the animals are rehabilitated and tested as adults (Lat et al, 1961; Cowley and Griesel, 1965; Novakova, 1966; Frankova and Barnes, 1968; Levitsky and Barnes, 1968; Hsueh et al, 1973; Simonson et al, 1973). On the other hand, an increase in voluntary activity has also been reported following

nutritional deprivation (Siegel and Steinberg, 1949; Finger 1951; Cornish and Mroskovsky, 1965; Hughes, 1965; Guthrie, 1968).

The methodology employed in the various studies, as also the severity and timing of undernutrition varies, making interpretation of the results difficult.

In the present experiment, an attempt was made to study the activity level of male rats rehabilitated for 5-6 weeks after being subjected to neonatal and/or postweaning undernutrition.

Males from Experiment 1(a) were taken at the age of 16 weeks. They were fed ad libitum throughout the period of study. Open field activity was measured in terms of the number of squares crossed during the 3 minutes of the test period (cf Materials and Methods) and activity in the activity wheel in terms of the number of rotations of the wheel during the 10 minutes of the test period (cf Materials and Methods). The results are presented in Tables 26 and 28 respectively.

A scruitiny of the data of Table 26 suggests the following :

(a) Undernutrition confined to the neonatal period had no significant effect on activity levels.

(b) Mild undernutrition induced during the neonatal period and continued in the postweaning period resulted in enhanced activity levels even 5 weeks after rehabilitation.

Table 26 : Activity in the open field after rehabilitation of male rats subjected to different degrees of preweaning and/or postweaning food restriction.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib controls (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± s.e.[a]				
		Average no. of squares trowersed in 3 mins				
4	45 - 50	49 ± 2	50 ± 1	51 ± 1	38 ± 1***	47 ± 1
8	35 - 45	46 ± 1	55 ± 1*	42 ± 4	38 ± 3*	45 ± 2
12	25 - 35	50 ± 2	56 ± 6	34 ± 2***	33 ± 2***	43 ± 2
Column mean[b]		48 ± 1	53 ± 2*	43 ± 2*	36 ± 1***	

1. Preweaning nutritional stress manipulated as shown in columns 1,2 and postweaning upto 11 weeks of age, as shown in columns 3,4,5 and 6.
Rehabilitation, 11-16 weeks of age.

a. Values based on 6-7 observations.
For tests of significance all groups compared with the best nourished group (litter size 4, weaning weight, 45-50 g, and fed ad lib after weaning). One, two and three asterisks respectively indicate significance at p values less than 0.05, 0.01 and 0.001.

b. Row means compared with values for litter size 4. Column mean compared with groups fed ad lib. after weaning.

(c) On the other hand, more severe undernutrition during the postweaning period resulted in a significant reduction in activity levels even after rehabilitation.

(d) With a more moderate degree of undernutrition this was evident when postweaning undernutrition was preceded by neonatal undernutrition.

An increase in activity as a consequence of undernutrition has led to the claim that this may increase the chance of encountering food (Cornish and Mroskovsky, 1965). While decreased activity is of interest in view of the common observation of a reduction in voluntary activity in undernourished individuals.

A summary of the various animal studies on the effects of undernutrition on activity is presented in Table 27. It suggests that an increase in activity is observed among the temporarily undernourished animals when tested without an intervening period of rehabilitation, while reduced activity levels result as a consequence of severe early undernutrition with an intervening period of rehabilitation before testing. Noteworthy is the study of Guthrie (1968) which employs a mild degree of undernutrition followed by rehabilitation. Here the animals showed an increased voluntary activity unlike similar animals which were subjected to severe early undernutrition.

Table 27 : Voluntary activity of undernourished animals as reported by various investigators.

Investigator	Period of restriction	Mode of restriction	Effect on voluntary activity
	Time of testing : after rehabilitation		
1. Lat *et al* (1961)	Suckling (0-3 weeks of life)	Litter size increased to 16 pups per mother	Decreased
2. Cowley and Griesel (1965)	Throughout the life of the mother	5% protein	Decreased
3. Novakora (1966)	Suckling	Premature weaning	Decreased
4. Frankova and Barnes (1968)	Suckling	5% protein diet to mother during lactation	Decreased
5. Guthrie (1968)	Suckling	8% protein diet to mother during lactation	Increased
6. Levitsky and Barnes (1968)	Suckling	2.5% protein diet to mother during lactation	Decreased
7. Hsueh *et al* (1973)	Suckling	50% of ad lib food intake to mother during lactation	Decreased
8. Simonson *et al* (1973)	Intrauterine and suckling	50% of ad lib food intake to mother during gestation and lactation	Decreased

contd...

Table 2 7 : contd.

Investigators	Period of restriction	Mode ofrestriction	Effect on voluntary activity
	Time of testing : during period of food restriction		
1. Siegel and Steinberg (1949)	Postweaning	Quantity reduced	Increased
2. Finger (1951)	Postweaning	Quantity reduced	Increased
3. Cornish and Mrosovsky, (1965)	Adult	3 days total starvation	Increased
4. Hunghes (1965)	Adult	Quantity reduced	Incrdased
5. Rajalakshmi and Ramakrishnan (1969(a))	Suckling	Litter size increased to 16 pups per mother	Decreased

In marked contrast to the above observations, animals severely undernourished group in early life displayed significantly greater activity as measured by rotations of the activity wheel even after rehabilitation (Table 28). This was accompanied by a reduction in the number of 'standing up 'reactions' and the time spent in grooming although the mean values in the latter case fall short of significance except in the severely restricted groups, presumably because of the large variations in the values observed.

The differences in results in the two test situations may be attributed to the difference in the nature of the two activities. While activity in the open field represents voluntary activity without a stressor, the situation in the rotating wheel represents a more stressful situation. The increased activity in this case may therefore represent heightened emotionality. Reports have been made of heightened response to electrical shock following rehabilitation from prenatal and/or neonatal undernutrition (Simonson et al, 1973; Levitsky and Barnes,1970; Smart et al, 1973; Frankova, 1973), more spillage of food in animals having restricted access to food after weaning (Barnes et al, 1968), an increased motor and defecation response to acoustal stimuli in animals of the 1st filial generation of rats subjected to protein deficiency (Cowley and Griesel, 1963), more defecation in animals rehabilitated after postweaning undernutrition (Kendrick,1973), decreased social responsiveness and increased aggressive behavior (Smart, 1982) deficits in motor co-ordination (Jordan,1982) and reduced capacity to effectively utilize environmental stimuli to make appropriate coping responses (Wiener and Levine, 1982) in perinatally undernourished animals when tested as adults.

Table 28 : Behavioral measures in an activity wheel of male rats subjected to different degrees of preweaning and/or postweaning food restriction[1].

Litter size	Weaning weight (g)	Postweaning diet as % ad lib controls (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± s.e.[a]				
		No. of rotations made by the wheel in 10 mins				
4	45 - 50	1.7 ± 0.2	2.8 ± 1.1	3.6 ± 0.4**	4.3 ± 0.4***	3.3 ± 0.3
8	35 - 45	2.1 ± 0.2	3.6 ± 0.8*	6.0 ± 1.3**	6.4 ± 0.6**	5.4 ± 1.1
12	25 - 35	3.5 ± 0.7	4.4 ± 0.5**	4.7 ± 0.4**	6.4 ± 0.3***	4.6 ± 0.3
Column mean[b]		2.5 ± 0.3	4.0 ± 0.5*	4.8 ± 0.5**	5.7 ± 0.5**	
		No. of standing up reactions made by the animal in 10 minutes				
4	45 - 50	22 ± 2	22 ± 3	18 ± 2	8 ± 2***	18 ± 2
8	35 - 45	26 ± 3	17 ± 2	13 ± 3*	7 ± 2***	16 ± 2
12	25 - 35	26 ± 3	21 ± 5	11 ± 2**	9 ± 2***	17 ± 2
Column mean		24 ± 1	20 ± 2	14 ± 1***	8 ± 1***	

contd...

Table 28 : contd.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib controls (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		Time spent in grooming (sec)				
4	45 - 50	49 ± 13	34 ± 11	33 ± 7	17 ± 3*	33 ± 5
8	35 - 45	47 ± 10	30 ± 11	43 ± 14	7 ± 3**	32 ± 5
12	25 - 35	54 ± 4	48 ± 10	28 ± 4	12 ± 5*	36 ± 4
Column mean		50 ± 5	38 ± 6	35 ± 5	12 ± 2***	

1. Preweaning nutritional stress manipulated as shown in columns 1, 2 and postweaning upto 11 weeks of age, as shown in columbs 3,4,5 and 6.
Rehabilitation, 11-16 weeks of age.

a. Values based on 6-7 observations.
For tests of significance all groups compared with the best nourished group (litter size 4, weaning weight, 45-50 g. and fed ad lib after weaning). One, two and three asterisks respectively indicated significance at p values less than 0.05, 0.01 and 0.001.

b. Row means compared with values for litter size 4. Column means compared with groups fed ad lib after weaning.

In conclusion, the present studies confirm previous findings that nutritional stress may result in reduced voluntary activity and increased activity under stress conditions.

Experiment -1(c)

Reproductive performance of female rats subjected to different degrees of preweaning and/or postweaning food restriction.

As mentioned earlier, the effects of malnutrition in the early life are believed to affect not only subsequent physical and behavioral development but also maternal behavior (Frankova, 1974). However, no impairment in reproductive performance has been found when rehabilitation precedes mating (Widdowson, 1967; Widdowson and Cowen, 1972; Radhakrishnan, 1966). In fact, moderate undernutrition in early life is associated with improved lactation performance in dairy animals (Allden, 1970).

The present studies were concerned with the effects of undernutrition during the neonatal and/or postweaning periods on gestation and lactation performance as well as maternal behavior.

The female rats described in Experiment 1(a) were mated with well fed male rats from the stock colony when they were 16 weeks of age.

Data were obtained on several indices of reproductive performance such as maternal food intake and weight change during gestation and lactation, the number of pups born, the number surviving atweaning, and growth rate of pups till weaning. Although some of the indices used such as food

utilization obviously lack precision, they are believed to be sufficiently valid for comparative purposes. The results are presented in Table 29.

No adverse effects of either undernutrition during the neonatal or postweaning periods on litter size were evident. On the contrary, the data suggest some increase in the same with moderate undernutrition during the neonatal period. The results do not suggest any tendency for resorption of the fetus in animals subjected to previous undernutrition. In all the groups average litter size compared well with the expected range of 6-10 in the stock colony.

At weaning, the litter size was similar in all the groups, indicating that neonatal mortality was not higher in the previously undernourished groups.

The mean weights of pups at birth were not significantly different in any of the groups subjected to early undernutrition except in the group well nourished neonatally but fed 66% of ad lib intake postweaning. The significantly lower row mean of groups fed 66 and 50% ad lib postweaning indicated the tendency of the groups to produce smaller progeny at birth. However, when total birth weight was considered, none of the undernourished groups did less well than the controls. In fact, mild neonatal undernutrition (litter size 8, weaning weight 35-45 g) seemed to result in a tendency for increased

Table 29 : Reproductive performance after rehabilitation of female rats subjected to different degrees of preweaning and/or postweaning food restriction[1].

Litter size	Weaning weight (g)	Postweaning diet as % ad lib controls (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± se[a]				
		mean no. of pups per litter at birth				
4	45 - 50	8.8 ± 0.7	8.1 ± 0.7	11.0 ± 0.5	8.3 ± 3.2	9.2 ± 0.7
8	35 - 45	11.4 ± 1.0	10.8 ± 0.9	10.4 ± 1.2	12.4 ± 1.2*	11.3 ± 0.5*
12	25 - 35	9.6 ± 0.7	9.3 ± 1.0	8.0 ± 1.6	10.0 ± 1.0	9.3 ± 0.5
Column mean[b]		9.9 ± 0.7	9.3 ± 0.5	9.9 ± 0.6	10.5 ± 0.9	
		mean no. of pups per litter at weaning				
4	45 - 50	8.0 ± 1.3	7.2 ± 0.4	6.8 ± 0.4	6.0 ± 1.9	7.1 ± 0.4
8	35 - 45	8.0 ± 0.4	8.3 ± 0.9	7.1 ± 2.9	10.8 ± 1.0	7.8 ± 0.6
12	25 - 35	7.9 ± 0.4	8.0 ± 1.0	7.5 ± 1.4	7.6 ± 1.0	7.8 ± 0.4
Column mean		7.9 ± 0.4	7.5 ± 0.4	7.2 ± 0.7	7.4 ± 0.8	

contd...

Table 29 : contd.

1	2	3	4	5	6	7
			per cent mortality			
4	45 - 50	15 ± 7	11 ± 4	38 ± 2**	23 ± 14	22 ± 4
8	35 - 45	31 ± 5	33 ± 6	39 ± 19	18 ± 1	30 ± 4
12	25 - 35	18 ± 3	15 ± 3	5 ± 4	24 ± 4	17 ± 2
Column mean		21 ± 3	19 ± 3	28 ± 6	22 ± 3	
			Mean weights of pups (g) : at birth			
4	45 - 50	6.08 ± 0.09	6.02 ± 0.006	5.10*** ± 0.11	6.37 ± 0.04	5.84 ± 0.05
8	35 - 45	6.12 ± 0.09	5.98 ± 0.06	5.68 ± 0.15	5.70 ± 0.09	5.98 ± 0.05
12	25 - 35	6.00 ± 0.07	6.23 ± 0.05	5.80 ± 0.05	5.60 ± 0.15	5.94 ± 0.03
Column mean		6.06 ± 0.05	6.11 ± 0.04	5.51*** ± 0.07	5.85* ± 0.07	
			At 7 days			
4	45 - 50	13.0 ± 0.3	13.2 ± 0.1	11.5 ± 0.2	17.0 ± 0.1***	13.4 ± 0.2
8	35 - 45	13.6 ± 0.1	14.0 ± 0.2	15.0 ± 0.4**	12.5 ± 0.2	13.7 ± 0.1
12	25 - 35	14.1 ± 0.4	14.5 ± 0.3	13.5 ± 0.4	11.8 ± 0.4	13.6 ± 0.2
Column mean		13.7 ± 0.2	13.9 ± 0.1	13.1 ± 0.2	13.3 ± 0.8	

contd...

Table 29 : contd.

1	2	3	4	5	6	7
			At 14 days			
4	45 - 50	24.8 ± 1.1	24.8 ± 0.3	26.0 ± 0.5	26.0 ± 0.2	25.4 ± 0.3
8	35 - 45	26.8 ± 0.2	27.8 ± 1.3	18.3 ± 0.5	21.0 ± 0.3	24.0 ± 0.7
12	25 - 35	26.4 ± 0.1	26.7 ± 1.2	25.5 ± 0.8	21.6 ± 0.9	25.2 ± 1.1
Column mean		26.1 ± 0.3	26.3 ± 0.6	23.9 ± 0.5**	22.5 ± 0.4**	
			At 21 days			
4	45 - 50	43 ± 5	45 ± 2	42 ± 2	44 ± 5	43 ± 1
8	35 - 45	46 ± 1	42 ± 4	36 ± 6	34 ± 2	40 ± 2
12	25 - 35	42 ± 1	43 ± 3	44 ± 4	37 ± 4	41 ± 1
Column mean		43 ± 1	43 ± 1	41 ± 2	38 ± 2	
			Total weights of pups (g) in the litter : at birth			
4	45 - 50	51 ± 5	49 ± 4	53 ± 4	51 ± 21	51 ± 3
8	35 - 45	70 ± 5*	64 ± 7	60 ± 7	71 ± 5*	67 ± 3
12	25 - 35	58 ± 4	58 ± 5	47 ± 10	55 ± 5	55 ± 3
Column mean		60 ± 3	53 ± 3	54 ± 4	60 ± 5	

contd...

Table 29 : contd.

1	2	3	4	5	6	7
			At 7 days			
4	45 - 50	108 ± 8	94 ± 5	80 ± 7	99 ± 26	94 ± 5
8	35 - 45	114 ± 5	101 ± 13	104 ± 39	134 ± 10	114 ± 7
12	25 - 35	103 ± 5	110 ± 10	98 ± 15	90 ± 14	101 ± 5
Column mean		108 ± 4	102 ± 5	92 ± 9	107 ± 9	
			At 14 days			
4	45 - 50	194 ± 5	177 ± 9	181 ± 8	154 ± 48	178 ± 7
8	35 - 45	220 ± 12	194 ± 3	136 ± 58	223 ± 12	199 ± 12
12	25 - 35	199 ± 8	201 ± 15	186 ± 28	164 ± 13	189 ± 8
Column mean		205 ± 8	191 ± 7	171 ± 15	181 ± 14	
			At 21 days			
4	45 - 50	318 ± 5	322 ± 14	287 ± 19	256 ± 50	311 ± 23
8	35 - 45	349 ± 20	297 ± 20	310 ± 15	359 ± 14*	315 ± 13
12	25 - 35	325 ± 12	330 ± 31	300 ± 36	266 ± 34	308 ± 14
Column mean		330 ± 13	318 ± 13	297 ± 12	297 ± 12	

contd...

Table 29 : contd.

1	2	3	4	5	6	7
		maternal weight gain (g) during gestation				
4	45 - 50	94 ± 5	86 ± 5.	66 ± 4**	91 ± 12	83 ± 4
8	35 - 45	104 ± 10	106 ± 5	81 ± 9	96 ± 12	96 ± 5
12	25 - 35	96 ± 3	78 ± 4	67 ± 8*	81 ± 4	82 ± 3
Column mean		97 ± 3	88 ± 4	72 ± 4	89 ± 5	
		wt. of pups born/g maternal wt. gain during gestation[c]				
4	45 - 50	0.58±0.08 (0.54)	0.56±0.05 (0.50	0.82±0.05** (0.51)	0.55±0.17 (0.41)	
8	35 - 45	0.70±0.03 (0.50)	0.61±0.06 (0.41)	0.76±0.05 (0.54)	0.78±0.07 (0.74)	
12	25 - 35	0.60±0.01 (0.49)	0.75±0.06 (0.63)	0.68±0.09 (0.65)	0.68±0.06 (0.53)	
		Food intake (g) during gestation				
4	45 - 50	392 ± 9	409 ± 2	409 ± 4	390 ± 14	
8	35 - 45	406 ± 11	403 ± 3	402 ± 8	404 ± 9	
12	25 - 35	409 ± 9	398 ± 3	384 ± 14	398 ± 9	

contd...

Table 29 : contd.

1	2	3	4	5	6	7
		Total tissueproduced/g food intake during gestation / during gestation				
4	45 - 50	0.24 ± 0.01	0.22 ± 0.01	0.16 ± 0.01***	0.23 ± 0.02	
8	35 - 45	0.25 ± 0.02	0.26 ± 0.01	0.21 ± 0.03	0.23 ± 0.03	
12	25 - 35	0.24 ± 0.02	0.20 ± 0.01*	0.17 ± 0.02**	0.20 ± 0.01*	
		Maternal weight loss (g) during lactation				
4	45 - 50	51 ± 6	43 ± 9	38 ± 10	69 ± 8	48 ± 5
8	35 - 45	43 ± 6	40 ± 3	53 ± 33	76 ± 11	52 ± 7
12	25 - 35	35 ± 2	43 ± 5	45 ± 11	39 ± 14	40 ± 4
Column mean		42 ± 3	42 ± 3	44 ± 8	58 ± 8	
		Food intake (g) during lactation				
4	45 - 50	604 ± 17	597 ± 9	594 ± 7	587 ± 25	696 ± 12
8	35 - 45	608 ± 7	603 ± 9	585 ± 16	628 ± 19	606 ± 10
12	25 - 35	615 ± 10	588 ± 24	598 ± 3	608 ± 4	602 ± 8
Column mean		609 ± 6	596 ± 5	592 ± 10	608 ± 14	

contd...

Table 29 : contd.

1	2	3	4	5	6	7
		Total tissue produced/g food intake during lactation/ during lactation				
4	45 - 50	0.44 ± 0.02	0.47 ± 0.01	0.42 ± 0.02	0.31 ± 0.06*	
8	35 - 45	0.53 ± 0.03	0.43 ± 0.02	0.32 ± 0.05*	0.45 ± 0.01	
12	25 - 35	0.51 ± 0.05	0.48 ± 0.04	0.43 ± 0.04	0.37 ± 0.05	

1. Preweaning nutritional stress manipulated as shown in column 1,2 and postweaning upto 11 weeks of age, as shown in columns 3,4,5 and 6, thereafter, all animals fed ad lib.

a. Values based on 3-7 litters or 25-50 pups.= For tests of significance all groups are compared with the best nourished group (litter size 4, weaning weight, 45-50 g, and fed ad lib after weaning). One, two and three asterisks respectively indicate significance at p values less than 0.05, 0.01 and 0.001.

b. Row means compared with values for litter size 4. column means compared with group fed ad lib after weaning.

c. Values in parenthesis are obtained after accounting for weight of pups eaten up by the mothers soon after birth.

total pup weight at birth and since the litter size of these groups is also slightly more, the smaller mean birth weights of these progeny indicate that the number of pups born is increased at the cost of individual weights of pups.

Maternal weight gain during gestation was similar in all groups except two, viz., the group well nourished neonatally and fed 66% ad lib postweaning and the group moderately undernourished neonatally (litter size 12, weaning weight 25-35 g) and fed 66% ad lib postweaning. The total weight of pups born/maternal weight gain during gestation remains significantly higher for the former but not for the latter. However, the total weight of pups surving at weaning/maternal weight gain during gestation was similar for all the groups suggesting the possibility that the animal that produced excess tissue inspite of a poor weight gain during gestation made up for it by eating some of the pups she produced.

The food intake of all the groups studied was similar. The efficiency of its utilization for tissue production as measured by tissue gained during gestation per unit food intake, however does not present a consistent picture. Certain undernourished groups show an impairment in efficiency of tissue production while others do not, raising the questions whether it is an interplay of several factors that causes this.

An analysis of the data on the mean weight of pups during lactation also does not present a clear picture. Some groups appear to emerge significantly different from controls at random at one week and not at another. However, the total weight of progeny in various groups at 7, 14 and 21 days of age is not significantly less than controls at any point. The cause for this apparent anomally can be attributed to the small differences in litter size in the various groups. For instance, the progeny of the group undernourished mildly (litter size 6, weaning weight 35-45g) neonatally and severely (50% ad lib) postweaning had a significantly lower ($p < 0.001$) mean weaning weight but the total weight at 21 days of progeny produced by this group was significantly higher ($p < 0.05$) than that produced by the controls. The mean litter size in the control group was 8.0 as against 10.8 in the experimental group. The maternal weight loss is not significantly different in any of the groups studied indicating that the mother is not functioning at the cost of her own body resources.

Food intake during lactation was similar for all the groups. Again, as in the case of gestation the utilization of food ingested for tissue production as measured by tissue

gained during lactation per unit food intake presents an apparently inconsistent pattern.

The overall picture suggests no impairment with regard to gestation and lactation performance as a result of early undernutrition followed by rehabilitation. The apparent impairment in food utilization for tissue production in some groups does not seem to bear any relation to the degree of nutritional stress experienced by the mother in early life.

Previous studies by Widdowson and Coworkers show that severe neonatal or postweaning deprivation followed by rehabilitation does not impair subsequent reproductive performance as judged by birth weight, weaning weight and number of pups produced once sexual maturity is reached. However, the latter is delayed as a result of early undernutrition and seems to depend on the weight of the animals so that at the time of delivery, the experimental groups are 18 weeks old as against the controls who are 11 weeks old (Widdowson and McCance, 1960; Widdowson et al, 1964; Widdowson, 1967; Widdowson and Cowen, 1972). In contrast, in our study, all females were mated at the age of 16 weeks so that the youngest animal, irrespective of early dietary history was 19-23 weeks old at the time of delivery. The body weights of the various groups, as given in Table 20, were not comparable at the time of mating. In a study by Widdowson (1967). Chronic deprivation of protein (7%) and/or calories (pairfed with the 7% protein group) delayed

puberty, reduced fertility and gave rise to a smaller litter at birth. However, more severe protein or caloriedeficiency, followed by rehabilitation had no carry over effects on reproductive performance. Similarly, Radhakrishnan (1966) found that feeding a protein free diet for four week intervals at different ages to female rats did not impair reproductive performance unless depletion was effected just prior to mating resulting in significantly lower body weights at the time of mating. All these studies employed severe undernutrition and found no impairment in reproductive performance if rehabilitation precedes mating. In dairy cattle, mild undernutrition has been reported to improve subsequent lactation performance, more so in the second and subsequent parities (Allden,1970). The present studies employed mild to severe early undernutrition but the results did not suggest any definite improvement or impairment in reproductive performance. However, these results are based on first parity ~~only~~ performance which is known to be highly variable.

Experiment - 1(d)

Maternal behavior of rats subjected to different degrees of preweaning and/or postweaning food restriction.

As mentioned earlier, nutritional stress in early life seems to have an adverse effdct on maternal behavior (Frankova, 1971, 1974; Massaro et al, 1974, 1977). On the other hand, the superior lactation performance of females undernourished in early life has also been reported (Crichton et al, 1959; Allden, 1970; Ura et al , 1972).

An attempt was therefore made in the present investigations to make a simultaneous study of both aspects. As described earlier, no adverse effects were found on the survival and growth of the progeny of animals undernourished in early life suggesting that their gestation and lactation performance was unimpaired.

The findings on maternal behavior are reported in this section.

As described elsewhere, maternal behavior was assessed by the method of Frankova (1974). The indices used were modified slightly and included total exploratory activity of the mother, latency before first contact with pups and the proportion of pups retrieved, measured on days 6 and 7 of lactation. The data obtained are presented in Table 30.

The total exploratory activity in the test situation on day 1 of testing (i.e. day 6 of lactation) was not affected by either neonatal or postweaning undernutrition singly. However, when postweaning undernutrition was superimposed on neonatal deprivation a tendency for increased exploration was seen. Although all the relevant groups do not show significantly greater values this trend is evident from the values given in Table 30.

On day 2, in trial (i) none of the groups displayed significantly different exploratory activity compared to controls. However, the trend for increased activity in the groups undernourished postweaning is evident from the fact that row mean of these groups is significantly greater than the row mean of the groups fed ad lib. This trend, however, disappeared in trial (ii).

Latency before first contact with pups on day 1 showed the same pattern as exploratory activity in the groups in which neonatal undernutrition had been followed by postweaning. The pattern persisted on day 2 in trial (i). In trial (ii), greater latency was shown even by groups normally nourished neonatally and fed 66 or 50% ad lib postweaning as well as by those subjected to mild or moderate neonatal (litter size 8 or 12, weaning weight 45-50 g) and mild to severe (80, 66 or 50% ad lib) deprivation postweaning. The effects of postweaning deprivation alone were aggravated when combined with neonatal

Table 30 : Maternal behavior of rats subjected to different degrees of preweaning and/or post-weaning food restriction.

Litter size	Weaning weight (g)	Postweaning diet as % ad lib controls (3-11 weeks)				Row mean[b]
		100	80	66	50	
1	2	3	4	5	6	7
		mean ± se[a]				
		Day 1 - Total exploratory activity (sec.)				
4	45 - 50	456 ± 31	412 ± 51	506 ± 39	434 ± 109	456 ± 22
8	35 - 45	530 ± 21	436 ± 34	532 ± 43	571 ± 31*	517 ± 18
12	25 - 35	472 ± 15	482 ± 22	568 ± 30*	559 ± 44	512 ± 16
Column mean[b]		486 ± 17	447 ± 20	533 ± 20	532 ± 32	
		Day 2 trial (i)				
4	45 - 50	345 ± 56	305 ± 19	422 ± 41	440 ±107	373 ± 25
8	35 - 45	373 ± 39	369 ± 19	373 ± 39	408 ± 80	380 ± 20
12	25 - 35	299 ± 7	462 ± 26	439 ± 64	450 ± 52	403 ± 22
Column mean		335 ± 20	385 ± 22	415 ± 25*	433 ± 35*	

contd...

Table 30 : contd.

1	2	3	4	5	6	7
			Day 2 trial (ii)			
4	45 - 50	925 ± 60	912 ± 57	959 ± 83	879 ± 61	924 ± 29
8	35 - 40	1042 ± 48	1006 ± 8	950 ± 67	971 ± 33	995 ± 20
12	25 - 35	856 ± 51	945 ± 84	903 ± 56	1091 ± 5	946 ± 55
Column mean		938 ± 35	954 ± 36	939 ± 35	998 ± 37	
			Latency before 1st contact with pup(sec.)			
			Day 1			
4	45 - 50	369 ± 32	203 ± 38**	398 ± 74	341 ± 160	324 ± 36
8	35 - 45	443 ± 55	286 ± 25	315 ± 74	541 ± 65*	404 ± 35
12	25 - 35	315 ± 39	432 ± 25	538 ± 56*	553 ± 49*	442 ± 31
Column mean		369 ± 27	317 ± 32	424 ± 44	496 ± 48*	
			Day 2 trial (i)			
4	45 - 50	201 ± 12	167 ± 17	210 ± 33	337 ± 164	221 ± 28
8	35 - 45	205 ± 22	254 ± 28	280 ± 34	352 ± 64*	272 ± 22
12	25 - 35	168 ± 10	306 ± 9	273 ± 42	418 ± 55**	287 ± 26
Column mean		187 ± 11	252 ± 18	246 ± 20*	376 ± 41***	

contd...

Table 30 : contd.

1	2	3	4	5	6	7
			Day 2 trial (ii)			
4	45 - 50	9 ± 3	10 ± 4	58 ± 6***	72 ± 6***	35 ± 8
8	35 - 45	9 ± 3	24 ± 2**	179 ± 3***	903 ± 80***	279 ± 98*
12	25 - 35	12 ± 3	32 ± 7*	321 ± 79***	991 ± 107***	296 ± 99
Column mean		10 ± 2	24 ± 4*	186 ± 40***	732 ± 129***	
			per cent pups retrieved Day 1			
4	45 - 50	53 ± 19	52 ± 15	50 ± 21	41 ± 38	50 ± 9
8	35 - 45	42 ± 22	83 ± 9	24 ± 16	7 ± 6***	40 ± 10
12	25 - 35	56 ± 7	41 ± 8	16 ± 11	9 ± 7***	46 ± 7
Column mean		49 ± 9	56 ± 7	32 ± 10	16 ± 9*	
			Day 2 trial (i)			
4	45 - 50	98 ± 3	100 ± 0	87 ± 7	56 ± 36	87 ± 7
8	35 - 45	100 ± 0	97 ± 4	92 ± 10	44 ± 23*	83 ± 8
12	25 - 35	100 ± 0	100 ± 0	67 ± 29	53 ± 22	84 ± 8
Column mean		99 ± 1	99 ± 1	83 ± 9	51 ± 12*	

contd...

Table 30 : contd.

1	2	3	4	5	6	7
			Day 2 trial (ii)			
4	45 - 50	100 ± 0	100 ± 0	75 ± 29	72 ± 22	88 ± 8
8	35 - 45	100 ± 0	97 ± 4	86 ± 9	57 ± 20*	85 ± 7
12	25 - 35	100 ± 0	100 ± 0	70 ± 13	40 ± 20*	81 ± 7
Column mean		100 ± 0	99 ± 1	76 ± 10	54 ± 11**	

1. Preweaning nutritional stress manipulated as shown in columns 1,2 and postweaning upto 11 weeks of age, as shown in columns 3,4,5 and 6; thereafter, all animals fed ad lib.

a. Values based on 3-7 observations
For tests of significance, all groups are compared with the best nourished group(litter size 4, weaning weight 45-50g, and fed ad lib after weaning), One, two and three asterisks respectively indicate significance at p values less than 0.05, 0.01 and 0.001.

b. Row means compared with values for litter size 4; column means compared with group fed ad lib after weaning.

undernutrition. Comparison of these values with those of corresponding groups on day 1 and day 2, trial (i), shows that the latency period decreases considerably in trial (ii) and this decrease is more in the control group than in the undernourished groups.

The percent of pups retrieved on day 1 were less than controls for the maximally undernourished groups. The pattern persisted on day 2 even though the percent retrieval was greater for all the groups on day 2. Values for trial (i) and (ii) were similar for corresponding groups.

The major observations that emerge from the foregoing are as follows :

(1) The tendency for increased exploratory activity with severe early undernutrition is seen on day 1. This contrasts with the behavior of males in the open field (ref. Experiment 1(a) but is more like the pattern inthe activity wheel, indicating the possibility that the maze for measuring maternal behavior is a more stressful situation for the animal than the open field.

(2) The pattern of exploratory activity changes with adaptation to the situation indicating that once the animal is familiar with the situation, it no longer finds it as stressful.

(3) First contact with pup was delayed as the severity of early undernutrition of the mother was increased.

(4) Mothers maximally undernourished in early life were also less inclined to retrieve their pups.

On the basis of these observations, it may be concluded that the heightened emotionality of the early undernourished mothers led to greater exploration and reduced inclination for pup retrieval. However, this may not necessarily result in crucial deficits in maternal behavior because those animals who did establish delayed contact spent about as much time with their pups as did the well nourished controls. This would account for the satisfactory growth of the latter demonstrating normal lactation. As reported in the previous section, total weaning weights of the undernourished groups were not significantly different from those of the controls.

In this connection, Frankova (1971, 1974) reported that when dams were fed low protein diets they spent less time with their pups and explored more during 3 minute tests on days 6,8 and 10 postpartum. On the basis of these decreases in two pup oriented behaviors, she concluded that low protein mothers displays a deficit in maternal attention towards their litters. In females reared by protein deficient mothers and fed a protein deficient diet upto 42 days of age, this deficit persists even after rehabilitation (Frankova, 1974).

On the other hand, Massaro and associates (1974) reported that when undisturbed low protein mothers were observed by photographic means during the 12 h dark period, they were found to spend more time with their young than similarly observed controls. The contradiction may be attributed to the differences in methodology. The former involved a situation novel for the animal in which the previously undernourished animal displayed greater excitement. The latter allowed for observation of the animal in its normal habitat.

In this connection, more recently, Galler and Propert (1981(a)) have reported the persistence of an increase in active nursing related behaviors in rats that were rehabilitated following intergenerational malnutrition. Further, when control pups were nursed by rehabilitated mothers and rehabilitated pups by control mothers (Gallers and Propert, 1981(b)) it was found that maternal factors were dominant in determing the amount of active nursing and that pup factors modified the maternal influence on other lactation oriented behaviors.

These observations when extended to the present study, explain the heightened emotionality in the test situation and suggest that in their ordinary cages, the undernourished animals spend at least as much, if not more, time nursing their young so that the latter receive sufficient milk for growth.

Experiment - 2

Comparative studies on two generations of rats fed diets simulating those consumed by different population groups with regard to reproductive performance, nutritional status and nitrogen balance during gestation and lactation.

In the studies just described, investigations were made of the effects of restricting food energy during critical periods using a qualitatively adequate diet.

In the human situation, however, thepattern is quite different. Poor people in developing countries subsist on diets inadequate in both quantity and quality throughout their lives. Studies were therefore made of the effects of feeding three typical diets consumed by selected population groups. One was the diet consumed by the poor people in Gujarat who are obviously on a low plane of nutrition as judged by growth and nutritional status. This diet, described as the diet of the low income group (LIG), is essentially similar to the diet consumed in several poor areas of the country except for differences in the staple consumed. The diet consumed by the upper class in Baroda (High Income Group or HIG) was taken as a diet representing that of the relatively more privileged sections of the population. An additional group was given a typical Western (British) diet as described by Greaves and Hollingsworth (1966) as the same is richer than the HIG diet in fat, sugar and protein.

Also, the feeding of diets varying in quality through several generations has been found to have cumulative effects on the development of the progeny in successive generations (Stewart, 1972(a), 1973). In the present studies, therefore, attempts were made to study the comparative growth and reproductive performance of animals fed the above diets for over two generations. Additional data were obtained on blood hemoglobin, serum protein and nitrogen balance during various stages of the reproductive cycle.

Young female rats 2-3 months of age from the stock colony were divided into three groups and fed the LIG, HIG and W diets for 4-6 weeks before mating and through gestation and lactation. The female progeny of these animals were continued on the respective diets and allowed to male at 12-14 weeks after weaning.

Data on the reproductive performance of animals fed the different diets is presented in Table 31.

In generation I, no differences were found between the different groups with regard to thenumber and total weight of pups born. The maternal weight gain during gestation was significantly more in the W group. The mortality rate was higher in the LIG compared to the other two groups and the growth ofpups in this group was slower as judged by their mean and total weight at 7, 14 and 21 days of age (Figs. 1,2).

Table 31 : Reproductive performance of rats fed diets consumed by different population groups.

Group		Maternal weight change (g)		No. of pups		Per cent morbidity	Total weight of pups (g)		Food intake (g)	Calorie intake
		Gestation	Lactation	At birth	At weaning		At birth	At weaning	Gestation + Lactation	Gestation + Lactation
LIG[b]	Go[a]	79 ± 5	-46 ± 6	8 ± 1	5 ± 1	42 ± 7	48 ± 4	135 ±17	760	2625
	G_1^a	59 ±12	-25 (-12,-29)	8 ± 1	6 (5,6)	36 (33,38)	47 ± 3	157 (140,174)	700	2415
HIG[b]	Go	94 ±10	-13 ± 4	9 ± 1	8 ± 2	11 ± 9	50 ± 6	249 ± 34	900	3320
	G_1	74 ± 4	-15 (-11,-19)	9 ± 1	8 (7,8)	17 (13,20)	51 ± 6	277 (266,288)	800	2960
W[b]	Go	106 ± 9	+2 ± 4	8 ± 1	7 ± 1	10 ± 5	50 ± 8	303 ± 34	1100	4100
	G_4	90 ± 5	-3 (+1,-6)	7 ± 1	7 (6,7)	14 (14,17)	39 ± 5	296 (276,315)	1000	3700

FIG.1.GROWTH CURVES OF PROGENY OF MOTHERS FED DIETS CONSUMED BY DIFFERENT POPULATION GROUPS: MEAN BODY WEIGHTS.

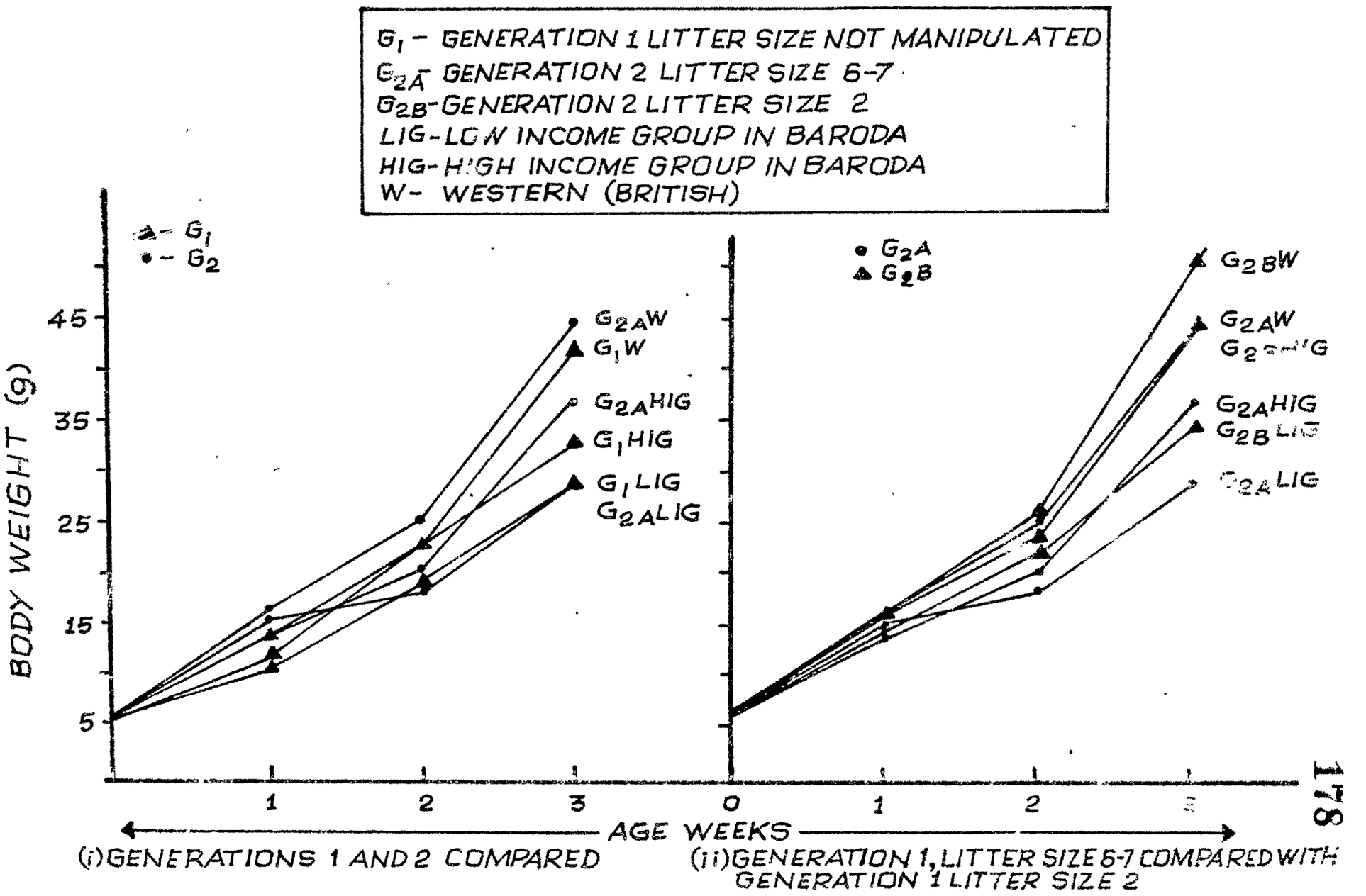
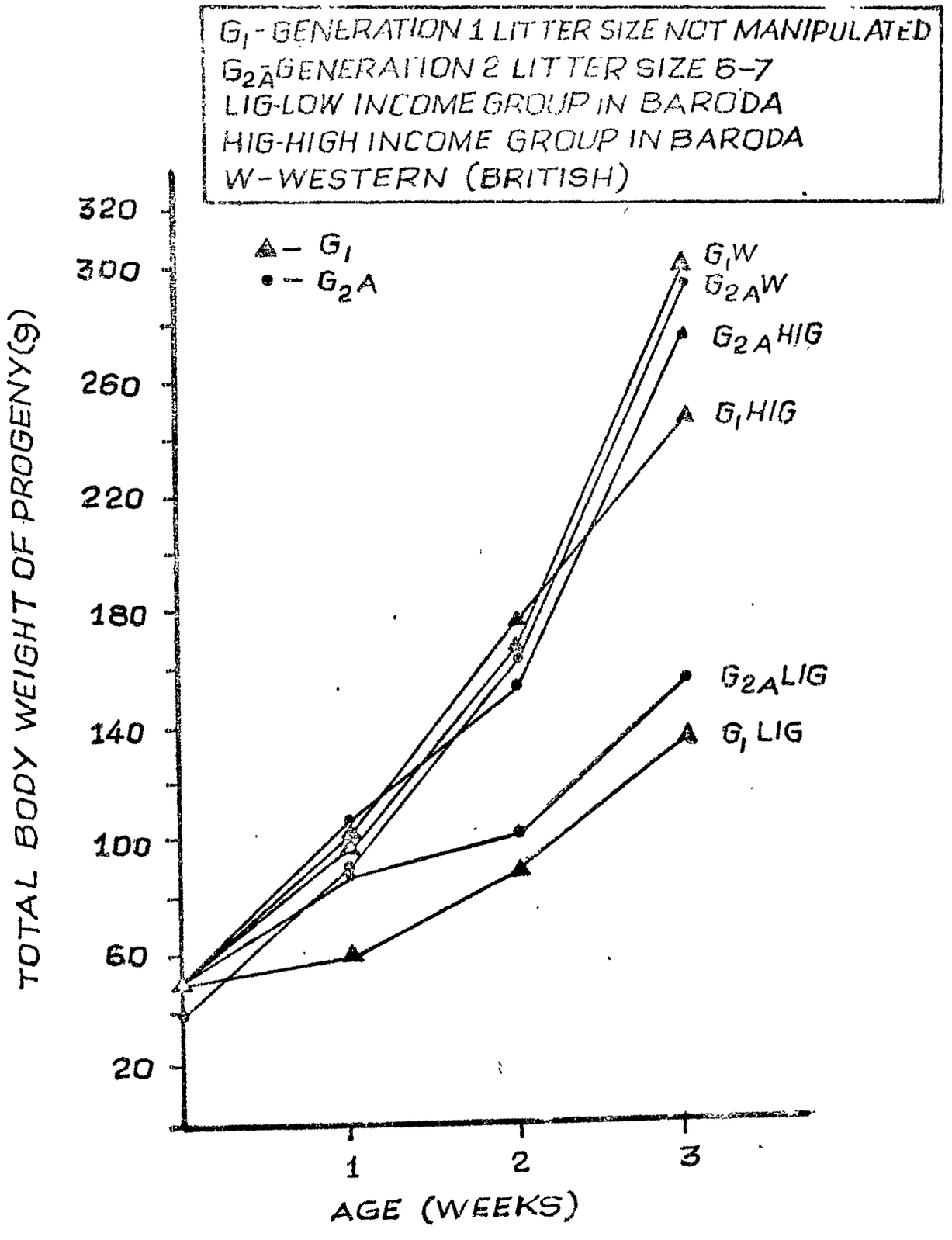


FIG-[illegible]GROWTH CURVES OF PROGENY OF MOTHERS FED DIETS CONSUMED BY DIFFERENT POPULATION GROUPS: TOTAL BODY WEIGHTS.



HIG and W group progeny did not differ significantly with regard to total weight of the progeny. But the mean weight in the W group was more than in the HIG group because of some differences in littersize. Maternal weight loss during lactation was maximum in the LIG group, followed by the HIG group. The W group gained some weight suggesting that this diet protects to some extent against the nutritional stress of lactation. The net tissue produced in absolute terms and relative to food intake and energy intake was less in the LIG group than in the HIG and W groups which did not differ from each other.

The female progeny of these groups were maintained on the respective diets and mated subsequently. Data on their reproductive performance are also given in Table 31. It can be seen that the pattern inthe two generations remained essentially similar. Reducing the nutritional stress of lactation by limiting litter size to two resulted in reducing the differences between the different groups (Fig. 2(ii)) but the pattern persisted, and at 21 days of age, the mean weight of the pups followed the order LIG < HIG < W. Maternal weight loss during lactation also followed the same pattern.

Blood hemoglobin and serum protein were assayed initially, on the 14th day of gestation and on the 12th day of lactation for both generations and the results are presented in Tables 32, 33. The results suggest that differences between the

Table 32 : Changes in bloodhemoglobin during gestation and lactation in rats fed diets consumed by different population groups.

	LIG[a]	HIG[a]	W[a]
		mean ± se	
		Blood hemoglobin g/dl	
		Generation 0	
Initial	11.4 ± 0.3 (104)	11.3 ± 0.2 (103)	11.0 ± 0.4 (
Gestation	10.9 ± 0.4 (99)	11.0 ± 0.6 (100)	11.0 ± 0.4
Lactation	9.2 ± 0.4** (85)	10.8 ± 0.3 (100)	10.8 ± 0.3
		Generation 1, litter size 6-7	
Initial	11.0 (80) (11.1,10.9)	12.7 (93) (12.3,13.1)	13.7 (13.8,13.5)
Gestation	10.7 (80) (10.6,10.8)	12.3 (92) (12.1,12.4)	13.3 (13.5,13.0)
Lactation	10.0 (78) (10.0,9.9)	11.5 (90) (11.3,11.6)	12.8 (12.9,12.7)
		Generation 1, litter size 2	
Initial	10.9 (85) (10.5,11.2)	12.1 (96) (12.2,11.9)	12.8 (13.1,12.5)
Gestation	10.6 (83) (10.7,10.8)	11.7 (91) (11.9,11.5)	12.8 (13.0,12.6)
Lactation	10.1 (82) (10.1,10.1)	11.3 (92) (11.4,11.1)	12.3 (12.5,12.1)

contd...

Table 32 :- contd.

	LIG[a]	HIG[a]	W[a]
	All groups combined		
Initial	11.2 ± 0.2 (93)	11.8 ± 0.3 (98)	12.0 ± 0.5
Gestation	10.8 ± 0.2 (91)	11.5 ± 0;3 (97)	11.9 ± 0.4
Lactation	9.6 ± 0.3*** (83)	11.1 ± 0.2 (96)	11.6 ± 0.4
	Values for non-pregnant animals corresponding to the stages indicated.		
Initial	11.0 ± 0.2 (85)	12.5 ± 0.3 (96)	13.0 ± 0.3
Gestation	11.1 ± 0.2 (85)	12.5 ± 0.3 (105)	13.1 ± 0.2
Lactation	10.9 ± 0.2 (83)	12.4 ± 0.3 (95)	13.1 ± 0.2

Values in parentheses alongside indicate per cent of Western values. One, two and three asterisks indicate values significantly different from corresponding initial values at p less than 0.05, 0.01 and 0.001.respectively.

a. LIG - Low Income Group in Baroda
HIG - High " " " "
W - Western (British

Table 33 : Changes in serum protein during gestation and lactation in rats fed diets consumed by different population groups.

	LIG[a]	HIG[a]	W[a]
	Serum protein g/dl		
	Generation 0		
Initial	6.90 ± 0.23 (102)	6.83 ± 0.57 (101)	6.76 ± 0.13
Gestation	6.80 ± 0.23 (99)	6.88 ± 0.17 (100)	6.90 ± 0.25
Lactation	6.45 ± 0.31 (95)	6.76 ± 0.28 (100)	6.78 ± 0.17
	Generation 1, litter size 6-7		
Initial	6.60 (95) (6.31,6.89)	7.05 (101) (7.10,7.00)	6.95 (7.00,6.90)
Gestation	6.40 (91) (6.10,6.71)	6.90 (98) (7.00,6.80)	7.01 (7.08,6.94)
Lactation	6.40 (96) (6.08,6.72)	6.64 (99) (6.78,6.50)	6.70 (6.70,6.70)
	Generation 1, litter size 2		
Initial	6.65 (92) (6.50,6.80)	7.08 (98) (6.88,7.18)	7.20 (7.21,7.19)
Gestation	6.70 (96) (6.51,6.89)	6.85 (98) (6.71,6.98)	7.00 (6.87,7.13)
Lactation	6.38 (93) (6.30,6.46)	6.72 (98) (6.62,6.82)	6.85 (6.80,6.70)

contd...

Table 33 : contd.

	LIG[a]	HIG[a]	W[a]
		All groups combined	
Initial	6.76 ± 0.15 (98)	6.95 ± 0.38 (100)	6.92 ± 0.10
Gestation	6.63 ± 0.15 (96)	6.88 ± 0.08 (99)	6.94 ± 0.13
Lactation	6.43 ± 0.14 (95)	6.71 ± 0.12 (99)	6.78 ± 0.09
	Values for non-pregnant animals corresponding to the stages indicated.		
Initial	6.70 ± 0.08 (95)	7.10 ± 0.06 (100)	7.08 ± 0.07
Gestation	6.58 ± 0.03 (93)	6.92 ± 0.07 (98)	7.10 ± 0.13
Lactation	6.58 ± 0.12 (92)	7.08 ± 0.04 (99)	7.13 ± 0.09

Values in parentheses alongside indicate per cent of Western values. One, two and three asterisks indicate values significantly different from corresponding initial values at p less than 0.05, 0.01 and 0.001 respectively.

a. HIG - High Income Group in Baroda
LIG - Low " " " "
W - Western (British).

different groups were mainly due to those in the nutritional adequacy of the current diets for meeting the stress of reproduction rather than due to the early dietary history of the animals. No conclusive evidence of 'generational effects' was found within the span of the present studies.

Since neither litter size nor the previous nutritional history of the animal appeared to have a significant effect on the changes observed, the data for each dietary group were combined.

Blood hemoglobin did not register any significant change with the progress of gestation in any of the groups studied. This is in contrast with the pattern reported in women where a decrease in hemoglobin has been found with the progress of pregnancy (Shankar,1962; Thomson and Hytten,1966; Rajalakshmi and Ramakrishnan, 1969(b)). The difference between results on animals and humans may also be because of the hemodilution associated with pregnancy in women. During lactation, hemoglobin was found to fall in the LIG group but not in the HIG and W groups. This fall in the LIG group appears to be a result of the stress of lactation and not the due to the prolonged feeding of the diet as the non-pregnant controls at corresponding points do not show the same decrease. A scruting of the pattern in individual animals (Table 34) indicated that all animals of the LIG group but not of the HIG and W groups showed a decrease during lactation.

Table 34 : Longitudinal data on bloodhemoglobin and serum protein during gestation and lactation in females fed LIG, HIG and W diets.

Diet	Animal No.	Blood hemoglobin g/dl			Serum protein g/dl		
		Initial	Gestation	Lactation	Initial	Gestation	Lactation
1	2	3	4	5	6	7	8
LIG	Go 1	11.6	11.4	8.7	7.0	6.8	6.3
	Go 2	10.4	9.8	9.3	7.0	7.2	6.7
	Go 3	10.9	10.8	9.5	6.2	6.2	5.8
	Go 4	12.0	11.8	10.5	6.75	7.3	7.0
	Go 5	11.9	10.8	8.3	6.8	6.5	-
	G1 1	10.5	10.4	10.1	6.5	6.5	6.3
	G_1 2	11.2	10.8	10.1	6.8	6.9	6.5
	G_1 3	11.1	10.8	10.0	6.3	6.1	6.1
	G_1 4	10.9	10.6	9.9	6.9	6.7	6.7
mean ± se		11.2 ± 0.2	10.8 ± 0.2	9.6 ±0.3	6.75 ±0.15	6.63 ±0.15	6.43 ±0.14
HIG	Go 9	12.4	12.0	10.7	7.6	7.2	7.3
	Go 10	11.6	11.7	11.5	6.9	6.9	7.1
	Go 11	10,3	10.3	10.5	6.9	6.9	6.5
	Go 12	10.7	10.0	10.5	6.0	6.5	6.3
	G_1 9	12.2	11.9	11.4	6.9	6.7	6.6
	G_1 10	11.9	11.5	11.1	7.2	7.0	6.8
	G_1 11	12.3	12.1	11.3	7.1	7.0	6.7
	G_1 12	13.1	12.4	11.6	7.0	6.8	6.5

contd...

Table 34 : contd.

1	2	3	4	5	6	7	8
mean ± se		11.8 ± 0.3	11.5 ± 0.3	11.1 ± 0.2	6.95 ± 0.38	6.88 ±0.08	6.71 ±0.12
W	Go 15	12.0	10.3	11.5	7.1	6.9	6.8
	Go 16	10.0	10.2	10.0	6.9	7.3	7.1
	Go 17	11.7	11.9	11.1	6.5	6.1	6.6
	G_1 17	13.1	13.0	12.5	7.2	6.9	6.8
	G_1 18	12.5	12.6	12.1	7.2	7.1	6.9
	G_1 19	13.8	13.5	12.9	7.0	7.1	6.7
	G_1 20	13.5	13.0	12.7	6.9	6.9	6.7
mean +_ se		12.0 ± 0.5	11.9 ± 0.4	11.6 ± 0.4	6.92 ±0.10	6.94 ±0.13	6.78 ±0.09

Serum protein did not show any significant decrease with either gestation or lactation (Table 33) although the pattern, especially when the change in individual animals is considered (Table 34)is suggestive of some change.

In this connection, in another study in this laboratory (Dave,1980), the following pattern for blood hemoglobin and serum protein in stock diet fed animals during gestation and lactation was found :

	non-pregnant	late pregnancy	Post partum	3 weeks lactation
hemoglobin g/dl	11.3	9.4	8.8	10.4
Serum protein g/dl	5.6	4.1	4.5	4.6

This pattern differs from that obtained in the present study. The difference could be because in the present study, the estimations were done at mid-pregnancy whereas in the above study they were done in late pregnancy. A progressive fall in hemoglobin with the progress of gestation is indicated from the following data on women (Dave, 1980) which are typical of data obtained by many investigators (Shankar, 1962; Venkatachalam, 1962; NIN Annual Report, 1969; Rajalakshmi and Ramakrishnan,1969(b); Decker et al, 1977).

	non-pregnant	1st trimester	2nd trimester	3rd trimester	post partum	6 months lactation
hemoglobin g/dl	11.7	11.8	10.9	10.3	11.0	11.3
serum protein g/dl	6.6	6.6	6.1	6.1	6.2	6.5

The picture may also be complicated by seasonal variations in the pregnancy response on the basis of a recent study (Tevves et al, 1981), investigating the effects of seasonal variations on various blood parameters of European pigs brought up in tropical climates. The results obtained inthis study are presented below :

Season	Initial	Gestation	Lactation
	Blood hemoglobin g/dl		
hot and dry	13.5	12.3	11.3
rainy	12.5	12.6	11.8
cool	12.6	13.3	12.0
dry	12.1	12.3	12.3
	Serum protein g/dl		
hot and dry	7.50	7.59	7.59
rainy	7.35	7.30	6.85
cool	7.58	7.41	7.35
dry	7.60	7.64	7.55

Nitrogen balance studies were conducted initially, between the 14th-17th day of gestation and between the 12th-18th day of lactation. Results are presented in Tables 35-38 and individual values, in Table 39.

The inttial food and calorie intake were similar in all the groups but food nitrogen was significantly more in the HIG group than in the LIG group and in the W group than inthe HIG group. Food, calorie and nitrogen intake increased during gestation and more so during lactation.

Initial weight gain was comparable in the HIG and W groups but was less in the HIG group so that the efficiency of utilization was also less in this group initially. However, during gestation, the weight gains were similar in all the group. Noteworthy here is the improvement in the efficiency of of feed utilization of the LIG group during gestation. During lactation, the W group gained more weight than the LIG and HIG groups. Efficiency of food utilization was also more. The higher efficiency of food utilization during gestation was not maintained during lactation in the LIG and HIG groups, perhaps because requirements for pup metabolism are more than for fetal metabolism.

Apparent digestibility was maximum in the W group, followed by the HIG and LIG groups inthat order. Some increase in digestibility during gestation and lactation was indicated.

Table 35 : Food intake and utilization during gestation and lactation in rats fed diets consumed by different population groups.

	LIG[1]		HIG[1]		W[1]	
	Go^2	G_1^2	Go	G_1	G_1	Go
1	2	3	4	5	6	7
			mean ± se[3,4]			
			Food intake (g)			
1	38 ± 4	30 ± 10	29 ± 10	31 ± 1	34 ± 2	32 ± 2
G	39 ± 1^{ch}	38 ± 1^{di}	43 ± 5^{j}	42 ± 4^{k}	55 ± 2^{cl}	51 ± 1^{dm}
L	109 ± 6^{h}	75 ± 4^{fi}	94 ± 2^{j}	72 ± 3^{k}	99 ± 10^{i}	92 ± 2^{fm}
			Calorie intake (Kcal)			
1	135 ± 14	106 ± 8	118 ± 41	127 ± 4	137 ± 6	128 ± 9
G	160 ± 4^{ch}	133 ± 4^{di}	177 ± 20^{j}	173 ± 17^{k}	219 ± 10^{cl}	203 ± 5^{dm}
L	383 ± 21^{h}	261 ± 15^{fi}	329 ± 9^{j}	296 ± 11^{k}	395 ± 39^{l}	368 ± 6^{fm}
			Weight gain (g)			
1	8 ± 1^{jl}	6 ± 1^{adKm}	13 ± 2^{no}	13 ± $1^{a\ pg}$	13 ± 1^{r}	18 ± 1^{bs}
G	16 ± 1^{j}	16 ± 1^{k}	22 ± 2^{n}	27 ± 2^{ep}	23 ± 1^{r}	21 ± 1^{s}
L	15 ± 2^{hl}	20 ± 3^{m}	24 ± 3^{ho}	25 ± 4^{ig}	62 ± 2^{hr}	48 ± 9^{is}

contd...

Table 35 : contd.

1	2	3	4	5	6	7
			Weight gain per 100 Kcal			
1	5.9	5.7	11.0	10.2	9.5	10.2
G	10.0	12.0	12.4	15.6	10.5	10.3
L*	3.9	7.7	7.3	8.4	15.7	13.0

1. LIG - Low income group in Baroda; HIG - High income group in Baroda; W - Western, (British).
2. Go - gestation;0; G_1 - generation 1;
3. Values based on 3-6 observations. Values marked with the same letter are significantly different from each other at p less than 0.05.
4. All values are for 3 days estimates.

* Weight change of mother + weight gain of pups.

Table 36 : Nitrogen retention during gestation and lactation in rats fed diets consumed by different population groups.

	LIG[1]		HIG[1]		W[1]	
	Go	G_1	Go	G_1	Go	G_1
1	2	3	4	5	6	7
			mean ± se[3,4]			
			Food nitrogen (mg)			
1	607 ± 35^{aj}	491 ± 15^{bl}	1041 ± 155^{am}	882 ±142^{bo}	1384 ± 149^{ap}	1218 ± 4^{bq}
G	650 ± 65^{dk}	578 ± 19^{cl}	1358 ± 156^{dn}	1329 ± 134^{eo}	1970 ± 90^{dp}	1842 ± 43^{eq}
L	1534± 114^{gjk}	1250± 73^{hl}	2532 ± 66^{gmn}	802 ± 13^{f}	3590 ± 356^{gp}	3369 ± 76^{hq}
			Fecal nitrogen mg			
1	264 ± 16^{aj}	205 ± 9^{k}	360 ± 51^{n}	321 ± 52^{o}	474 ± 93^{aq}	342 ± 12^{t}
G	258 ± 11^{bj}	239 ± 15^{cl}	444 ± 48^{bm}	466 ± 49^{cp}	534 ± 18^{r}	432 ± 39^{s}
L	470 ± 46^{deij}	436 ± 33^{fgkl}	888 ± 92 ^{dmn}	737 ± 61^{fop}	711 ± 49^{eql}	874 ± 53^{gst}
			Urine nitrogen Mg			
1	257 ± 12	240 ± 12	401 ± 35	412 ± 28	781 ± 145^{an}	768 ± 28^{bp}
G	147 ± 30^{dj}	79 ± 14^{ek}	501 ± 76^{dl}	507 ± 67	1076 ± 27do	1172 ± 80^{ep}
L	628 ± 119^{gj}	340 ± 13^{hk}	1160 ± 75^{gl}	1108 ± 138^{hm}	2386 ± 316^{gno}	2161 ± 128^{hp}

contd...

Table 35 : contd.

1	2	3	4	5	6	7
			Nitrogen retained mg			
1	86 ± 26[d]	46 ± 10[ae]	194 ± 55[f]	112 ± 12[ag]	130 ± 20[h]	109 ± 18[i]
G	308 ± 5[d]	254 ± 6[e]	411 ± 175[f]	356 ± 67[g]	360 ± 54[h]	237 ± 49[i]
L	437 ± 50[d]	474 ± 42[e]	484 ± 31	550 ± 40[g]	493 ± 21[h]	388 ± 54[i]

1. LIG - Low income group in Barǿda; HIG - High income group in Baroda; W - Western (British).
2. Go - Generation 0; G_1 - Generation 1;
3. Values based on 3-6 observations. Values marked with the same letter are significantly different from each other at p less than 0.05.
4. All values are for 3 day estimates.

Table 37 : Apparent digestibility, per cent absorbed N retained, weight gain per g N and estimate of per cent protein in tissue gained during gestation and lactation in rats fed diets consumed by different population groups.

	LIG[1]		HIG[1]		W[1]	
	Go	G_1	Go	G_1	Go	G_1
1	2	3	4	5	6	7
			mean ± se[3,4]			
			Apparent digestibility			
1	55 ± 2[a]	57 ± 1[b]	63 ± 2[a]	64 ± 1[b]	70 ± 2[a]	72 ± 1[b]
G	60 ± 3	58 ± 2[e]	67 ± 1[d]	65 ± 1[e]	73 ± 1[d]	74 ± 2[e]
L	68 ± 3	63 ± 3	63 ± 3[g]	66 ± 1[h]	75 ± 1[g]	77 ± 3[h]
			per cent absorbed nitrogen retained			
1	23 ± 6[a]	14 ± 3[b]	30 ± 5	25 ± 5	13 ± 4	13 ± 3
G	74 ± 4[a]	78 ± 3[b]	31 ± 1	41 ± 6	25 ± 3	17 ± 4
L	42 ± 6[a]	58 ± 3[b]	29 ± 2	28 ± 8	18 ± 2	13 ± 5
			weight gain (g) per g N retained			
1	181 ± 70[e]	178 ± 43[fh]	87 ± 20	124 ± 15[h]	128 ± 32	136 ± 30
G	52 ± 3[e]	61 ± 3[f]	78 ± 30	80 ± 15	67 ± 8	96 ± 16
L	36 ± 2[ae]	49 ± 7[g]	50 ± 8[b]	47 ± 8[dh]	115 ± 18[ab]	121 ± 10[ed]

contd...

Table 37 : contd.

1	2	3	4	5	6	7
	estimate of per cent protein in tissue gained					
1	5.7 ± 1.7[a]	4.7 ± 1.0[f]	9.2 ± 2.0	5.4 ± 1.0	6.2 ± 1.4	5.7 ± 0.9
G	12.0 ± 0.7	10.3 ± 0.5	11.0 ± 3.9	8.7 ±1.4	9.7 ± 1.1[e]	6.9 ± 1.1
L	18.1 ± 2.0[af]	15.1 ± 1.3[cg]	13.3 ± 2.4[b]	14.5 ± 2.6[d]	5.0 ± 0.2[bef]	5.2 ± 0.4[edg]

1. LIG - Low ₹ income group in Bafoda; HIG - High income group in Baroda; W - Western (British).
2. Go - generation 0, G_1 - generation 1;
3. Values based on 3-6 observations. Values marked with the same letter are significantly different from each other at p less than 0.05.
4. All values are for three day estimates.

Table 38 : Values during gestation and lactation in rats fed diets consumed by different population groups expressed as per cent of pregestation (initial) values.

	LIG[a]		HIG[a]		W[a]	
	Go[b]	G_1[b]	Go	G_1	Go	G_1
	(Gestation/pre-gestation) 100					
Weight gain per 100 Kcal	169	210	113	153	111	101
N retained	358	552	212	318	277	317
apparent digestibility per cent absorbed	109	102	106	102	104	103
N retained	322	557	103	164	192	131
estimate of percent protein in tissue gained*	211	219	116	161	157	121
	(Lactation/pre-lactation)100					
Weight gain per 100 Kcal	66	135	66	82	165	127
N retained	508	1030	249	491	379	356
apparent digestibility per cent absorbed	124	111	100	103	107	107
N retained	183	414	97	112	139	100
estimate of percent protein in tissue gained*	318	321	145	269	81	91

a. LIG - Low income group in Barida. HIG - High income group in Baroda ; W - western (British).

b. Go - generation 0, G_1 - generation 1.

Table 39 : Nitrogen balance at different stages of the reproductive cycle of females fed LIG, HIG and W diets.

weight gain g		Food N (mg)		Urine N (mg)		Feces N (mg)		mg N retained	
I	II	I	II	I	II	I	II	I	II
1	2	3	4	5	6	7	8	9	10
Low Income Group									
Initial									
8	6	559	505	289	268	229	222	+41	15
8	5	508	485	228	256	230	210	+50	19
6	8	570	440	293	206	267	173	+10	61
8	6	477	543	218	294	215	229	+44	20
6	6	601	467	287	212	258	194	+56	62
7	6	632	443	258	204	256	175	+18	64
12	8	739	519	271	235	325	199	+143	85
10	4	770	525	211	245	332	238	+227	42
8 + 1	6±1	607 ± 38	491 ±15	257 ± 12	240 ± 12	264 ± 16	205 ± 9	86 ±27	46 ±10
Gestation									
14	16	462	582	228	106	230	233	295	243
14	14	711	533	124	64	284	220	303	249
16	15	647	603	63	89	276	225	308	259
18	17	693	595	135	55	243	177	315	265
19		739		160		259		318	
16 ± 1	16 ± 1	650 ± 65	578 ± 19	142 ± 30	79 ±14	258 ± 11	239 ± 15	308 ± 5	254 ± 6

contd...

Table 39 : contd.

1	2	3	4	5	6	7	8	9	10
Lactation									
19	18	1867	1090	948	323	441	362	478	405
11	15	1314	1237	626	373	321	437	366	422
14	15	1645	1287	767	333	531	446	347	508
15	22	1340	1386	472	330	524	499	402	557
17		1497		375		532		590	
15	20	1534	1250	628	340	470	436	366	361
± 2	± 3	± 114	± 73	±119	± 13	± 46	± 33	± 55	± 36
High Income Group									
Initial									
5	16		860	328	374	185	337	63	109
16	14	745	695	351	381	293	231	101	83
15	10	745	783	322	405	304	284	119	94
12	12	994	699	450	385	365	210	179	105
19	16	1352	791	522	385	463	287	367	119
13	12	1317	1542	432	542	550	537	335	161
13	13	1041	882	401	412	360	321	194	112
± 2	± 1	± 155	± 142	± 35	± 28	± 57	± 52	± 55	± 12
Gestation									
19	23	1049	1332	572	644	347	453	130	236
20	30	1236	1011	584	374	408	354	234	283
23	25	1520	1430	538	468	502	519	480	443
26	28	1628	1542	309	542	519	537	800	463
22	27	1358	1329	501	507	444	466	411	356
± 2	± 2	± 156	± 134	± 76	± 67	± 48	± 49	± 175	± 67

contd...

Table 39 : contd.

1	2	3	4	5	6	7	8	9	10
Lactation									
28	29	2558	2413	1351	1109	790	845	417	459
25	18	2650	2101	1097	878	1083	677	470	546
24	31	2540	2219	1094	1016	942	626	504	577
16	22	2380	2387	1099	1429	738	800	543	619
24	25	2530	2278	1160	1108	888	737	484	550
± 3	±4	± 66	± 85	± 75	± 138	± 92	± 61	± 31	± 40
Western Initial									
12	16	1224	1322	795	891	389	378	46	53
14	12	1224	1131	770	744	352	330	102	57
15	9	1159	1222	683	795	348	354	128	73
12	6	1159	1000	756	632	269	278	134	90
16	13	2207	1311	1467	811	596	370	144	130
15	13	1332	1202	224	718	946	352	160	132
10	15	1385	1241	770	753	416	339	199	149
	16		1313		798		331		164
13	13	1384	1218	781	768	474	342	130	109
± 1	± 1	± 149	± 41	±145	± 28	± 93	± 12	± 20	± 18
Gestation									
19	20	1782	1872	1023	1342	517	380	242	150
24	19	1812	1810	1029	1105	507	497	276	218
22	22	1964	1923	1074	1211	530	481	360	231
25	24	2207	1752	1102	1031	596	301	509	350

contd...

Table 39 : contd.

1	2	3	4	5	6	7	8	9	10
25		2083		1151		521		411	
23	21	1970	1847	1076	1172	534	432	360	237
± 1	± 1	± 90	± 43	± 27	± 80	± 18	± 39	± 54	± 49
Lactation									
56	32	3522	3200	2319	2242	740	886	463	388
63	37	3225	3510	2116	2415	645	744	464	351
64	59	3300	3411	2192	2081	627	921	481	409
65	62	4830	3355	3471	1906	869	946	490	503
62		3073		1832		678		565	
62	48	3596	3369	2386	2161	711	874	493	388
± 2	± 9	±356	± 76	±316	±128	± 49	± 33	± 21	± 54

Table 39: (contd.) Non-pregnant controls, estimates obtained at points corresponding to those of gestation and lactation in pregnant animals.

Weight gain (g)	Food N (mg)	Urine N (mg)	Fecal N (mg)	mg N retained
1	2	3	4	5
LIG				
Initial				
+ 6	505	268	222	15
+ 5	485	256	210	19
+ 4	525	245	238	42
+ 8	399	206	173	20
6 ± 1	479 ± 33	234 ± 16	211 ± 10	24 ± 7
Point corresponding to gestation value				
+ 8	551	259	248	44
+ 6	530	274	224	32
+ 9	572	284	265	23
+ 6	489	243	212	34
7 ± 1	536 ± 21	265 ± 11	237 ± 20	33 ± 5
Point corresponding to lactation value.				
+ 3	540	290	228	22
+ 2	603	356	217	30
+ 4	568	259	258	51
+ 5	528	285	211	32
4 ± 1	560 ± 20	298 ± 24	229 ± 15	34 ± 7
HIG				
Initial				
+ 10	783	405	284	94
+ 12	699	384	210	105

contd...

Table 39 : contd.

1	2	3	4	5
+ 11	702	337	264	98
+ 12	689	327	267	103
11 ± 1	725 ± 26	383 ± 17	2568 ± 18	104 ± 5
Point corresponding to gestation value				
+ 10	801	413	276	112
+ 13	825	376	268	161
+ 10	758	436	274	145
+ 13	792	379	258	121
+ 12	802 ± 13	423	266	103
12 ± 1		405 ± 13	268 ± 31	128 ± 12
Point corresponding to lactation value				
+ 8	780	310	291	179
+ 8	855	341	236	110
+ 10	766	330	270	150
+ 15	780	313	253	100
+ 12	753 ± 24	404	243	133
11 ± 2		340 ± 11	259 ± 52	134 ± 16
Western Initial				
+ 15	1241	753	339	149
+ 16	1322	891	378	53
+ 13	1311	811	370	130
+ 9	1222	795	354	73
13 ± 2	1274 ± 29	813 ± 34	360 ± 35	101 ± 27

contd...

Table 39 : contd.

1	2	3	4	5
Point corresponding to gestation value				
+ 14	1612	881	409	322
+ 2	1723	1133	489	101
+ 10	1522	1071	390	61
+ 16	1340	845	362	133
11 ± 4	1549 ± 95	983 ± 83	413 ± 22	154 ± 68
Point corresponding to lactation value				
+ 13	1710	1072	450	188
+ 17	1682	1022	458	202
+ 10	1610	1040	457	113
+ 16	1390	900	379	111
14 ± 2	1598 ± 85	1009 ± 49	436 ± 43	154 ± 28

The increase during lactation seems to be more for the LIG group but results were by no means conclusive. Nitrogen retention for the LIG animals was initially less than that for the HIG and Wgroup. It increased during gestation and lactation in all groups. The increase was maximum in the LIG groups and mg nitrogen retained by the LIG group during gestation and lactation was not different from that retained by the HIG and W groups.

Inspite of similar retentions, the observed deficits in growth of LIG progeny presented an apparent inconsistency. However, the growth of the progeny slows down mainly in the last week of lactation only, while the nitrogen balance studies were done from day 12-18 of lactation.

Percent of absorbed nitrogen retained by the three groups was initially similar. In the LIG group, it increased 3-5 fold during gestation but decreased during lactation though it continued to be significantly higher than initial. This increase was conspicuous by its absence in the HIG and W groups which did not differ significantly from each other. During lactation, the HIG group gained significantly more weight than the LIG group and the W group, significantly more than the HIG group. When weight gain per g nitrogen retained was calculated, initial and gestation values for all groups were not significantly different from each other though variability was large. However, during lactation, it was

significantly more for the W group than for the LIG and HIG groups. The percent protein in muscular tissue is approximately 15-20 while that in adipose tissue is approximately 2. Intermediate values give an idea of the proportion of muscular and and adipose tissue gained. Results obtained here indicated that the composition of tissue gained by LIG, HIG and W groups was similar initially and during gestation. However, during lactation, LIG and HIG groups laid down tissue containing significantly more nitrogen than that laid down by the W group, This suggested that the W group is more prone to adipose tissue accumulation during lactation than the other two groups.

Nitrogen balance data was alsoobtained for non-pregnant non-lactating animals of generation 1 at points corresponding to gestation and lactation of the mated animals (Table 40). No significant differences were observed at the 3 stages, confirming that the differences observed during gestation and lactation for the mated animals were due to the stress of reproduction and were not an effect ofage or prolonged treatment on the respective diets.

Comparison of nitrogen balance data obtained from rats of generation 0 and 1 is presented in Table 41. The data shows that the improvement in performance seen during gestation and lactation is more marked in generation 1 than in generation 0 in the groups fed low income and high income Gujarati diet but not in the group fed western group. Thus, some generational effect is evident.

Table 40 : Nitrogen balance data for non-pregnant, non-lactating animals of generation 1 fed diets consumed by different population groups at points corresponding to the stages specified.

LIG*			HIG*			G		
0	1	2	0	1	2	0	1	2
1	2	3	4	5	6	7	8	9
mean + se[a] : At points corresponding to the stage specified								
Food intake (g)								
32 ± 2	35 ± 1	37 ± 1	23 ± 1	25 ± 1	24 ± 1	35 ± 3	43 ± 3	44 ± 2
Calorie intake								
115 ± 8	123 ± 5	129 ± 5	94 ± 3	104 ± 2	98 ± 3	140 ± 3	170 ± 11	176 ± 9
Weight gain (g)								
6 ± 1	7 ± 1	4 ± 1	11 ± 1	12 ± 1	11 ± 2	13 ± 2	11 ± 4	14 ± 2
Weight gain per 100 Kcal								
5.2	5.7	3.1	11.7	11.5	11.2	9.3	6.5	8.0
Food nitrogen (mg)								
479 ± 33	536 ± 21	560 ± 20	725 ± 26	802 ± 13	753 ± 24	1274 ± 29	1549 ± 95	1598 ± 85
Fecal nitrogen (mg)								
211 ± 10	237 ± 20	229 ± 15	258 ± 18	268 ± 21	259 ± 52	360 ± 35	413 ± 22	436 ± 43

contd...

Table 40 : contd.

1	2	3	4	5	6	7	8	9
			Urine nitrogen(mg)					
234 ± 16	265 ± 11	298 ± 24	363 ± 17	405 ± 13	340 ± 11	813 ± 34	983 ± 83	1009 ± 44
			Nitrogen retained (mg)					
24 ± 7	33 ± 5	34 ± 7	104 ± 5	128 ± 12	134 ± 16	101 ± 27	154 ± 68	154 ± 28
			Apparent digestibility					
56 ± 1	57 ± 1	58 ± 1	65 ± 2	67 ± 1	66 ± 1	72 ± 1	74 ± 1	73 ± 1
			per cent absorbed nitrogen retained					
12 ± 6	11 ± 2	10 ± 2	23 ± 2	24 ± 2	30 ± 5	11 ± 2	13 ± 6	13 ± 2
			weight gain(g) per g N retained					
169 ± 5	176 ± 62	182 ± 46	98 ± 25	107 ± 30	102 ± 13	132 ± 35	129 ± 28	142 ± 33
			estimate of per cent protein in tissue gained					
5.1 ± 1.2	4.6 ± 2.1	6.1 ± 1.7	6.5 ± 1.5	5.7 ± 1.2	7.2 ± 0.8	5.6 ± 0.8	6.3 ± 1.2	6.9 ± 0.9

a. Values based on 3-4 observations per group.
LIG - Low income group in Baroda.
HIG - High income group in Baroda.
W - Western (British)

* Values designated 0, 1 and 2 correspond respectively to initial, gestation and lactation values for the animals in Tables 35, 36, 37.

Table 41 : Comparison of nitrogen balance in generations 0 and 1 during gestation and lactation of rats fed diets consumed by different population groups.

	LIG			HIG			W		
	1	G	L	1	G	L	1	G	L
				(G_1/Go) 100					
Weight gain per 100 Kcal	97	120	197	93	126	115	107	98	83
Nitrogen retained	53	83	109	58	87	114	84	66	79
Apparent digestibility	104	97	93	102	97	105	103	101	103
per cent absorbed N retained	61	105	138	83	132	97	100	68	72
weight gain per g N retained	98	117	136	143	103	94	106	143	105
estimate of per cent protein in tissue gained	82	86	83	57	79	109	92	71	104

LIG - Low income group in Baroda.

HIG - High income group in Baroda.

W - Western (British).

1 - initial

G - gestation

L - lactation

In summary, results of the experiment showed that :

(1) The gestation performance of the LIG group is affected slightly and the lactation performance more seriously, compared to the HIG and W groups. The W group was somewhat superior to the HIG group. The differences between HIG and W groups could be attributed to the differences in calorie intake since utilization of food for tissue production was similar in the two groups. The LIG group on the other hand, showed impaired food utilization which could, at least in part, be a result of the poorer quality of the diet.

No further deterioration was evident in the reproductive performance of the female progeny which was continued on the respective diets for a second generation.

When stress was reduced by reducing litter size to 2 animals per mother, effects on the LIG group persisted, even though severity was reduced.

(2) During gestation, neither bloodhemoglobin nor serum protein showed a change inany of the groups. During lactation, the former registered a fall while the latter rdmained unaffected in the LIG group. No effects were evident in the HIG and W groups.

The reduction, seemed to be due to the nutritional inadequacy of the diet at the time of reproductive stress and had little to do with prior nutrition.

(3) Initially, nitrogen retention was less in the LIG group as compared to the HIG and W groups. It increased inall the groups during gestation and lactation. The increase was maximum in the LIG group so that it retained as much nitrogen as the HIG and W groups. Percent of absorbed nitrogen retained was similar initially but was significantly more in the LIG group than inthe HIG and W groups during gestation and lactation.

The composition of tissue gained was similar for all the groups initially and during gestation but during lactation the tissue laid down by the LIG and HIG groups contained significantly more nitrogen than that laid down by the W group, indicating the latter's proneness for adipose tissue deposition.

Previous studies indicate an increased nitrogen retention in pregnant women as compared to non-pregnant controls on similar dietary protein intakes (Beaton, 1961; Rajalakshmi and Ramakrishnan, 1969(b); NIN Annual Report, 1972; Rao and Rao, 1974) and in pregnant rats as compared to non-pregnant ones (Rombaults et al, 1956; Spray, 1950; Naismith and Fears, 1971). The present studies suggested that the increase is more in the animals on a poor quality diet (LIG group) than in those on better quality diets (HIG and W groups). Naismith's group further reports that the capacity of the liver to produce urea is decreased as an adaptive mechanism to increase retention of nitrogen, due to reduction in the activity of liver alanine

amino transferase and argino-succinate synthetase. This results in availability of a greater proportion ofnitrogen for anabolic purposes. This effect has been traced to the increased progestdrone levels in pregnancy (Naismith and Fears, 1972; Naismith, 1973). However, progesterone decreases with a sharp increase in prolactin beginning almost 2 days before parturition (Tucker, 1974). The present study indicates a decrease in the percent absorbed nitrogen retained in the LIG group between gestation and lactation even though it remains significantly more than initial values. However, mode of adaptation during lactation remains largely unexplained. In this connection, Ellas and Dowling (1976) have reported structural changes. in the intestine of lactating rats making for enhanced absorption. In our study, however, increased absorption was not conclusively established, though some indication of such a process was seen especially in the LIG group.

Chronic marginal malnutrition in rats (Stewart, 1973) induced by feeding diets providing 5.8% protein calories resulted in significant deficits in the number of pups born to deficient mothers (Controls (C): 9.9; Protein deficient (P.D.): 8.6), in the total litter weights (C: 54.2g; PD: 41.0g) and in the number of small-for-dates born (C: 24; PD: 29.8) in the 1st generation. No further deficits were observed in the five subsequent generations on the same diets. The

undernutrition however, was more severe than that in the LIG group of the present study.

The persistence of differences between weaning weights of the progeny of the three groups in this study even when litter size was reduced to two pups per mother was also seen by Zeman and associates (1973) in protein deficient females. They attributed this to decreased DNA levels at birth. In this context, the importance of suckling and consequently that of the litter size on milk production must be mentioned. Moray and associates (1975) found that a dam suckling 12 to 15 pups produced twice as much milk as that suckling 6-9 young, while litters of three took less than one-third of the amount taken by litters of 6 or 9. Similarly Wurtman and Miller (1976) found no differences in body weight or composition of animals reared in very small litters (2-4 per mother) when compared to those reared in standard litters of 8 or 12.

Experiment - 3

Response of rats to variations in dietary protein content and to changes in the same as judged by growth, nutritional status, nitrogen balance and incorporation of a labelled amino acid in serum protein.

In the experiments previously described, studies were made of the adaptation of animals to diets low in food energy. Although a few studies have been made of the response of animals to diets varying in protein content very few studies have been made on the adaptation of animals switched from low to high protein diets and vice-versa.

Studies were made of the response of animals to variations in the protein content of the diet and changes in the same after prolonged adaptation to a selected level of dietary protein. In particular, comparisons were made of :

(a) animals fed 5, 10 or 20% protein throughout,

(b) animals fed 10% protein throughout and those switched to a 10% diet after maintenance on less (5%) or more (20%) protein,

(c) Animals fed a low (5%) protein diet throughout and those switched from a 5% to a 10 % protein diet,

(d) animals first fed a 5% protein diet, then switched to a 10% protein diet and switched back to a 5% diet and those fed a 5% protein diet throughout.

Weanling rats were fed 5, 10 or 20 percent protein diets ad libitum for 8 weeks. Thereafter, half the animals in the 5% and 20% groups were switched to a 10% diet. After a further period of 12 and 15 weeks respectively, two of the animals in the group switched from 5% to 10% protein and two in the 10% protein group were switched to 5% protein diet. All the animals were monitored upto 31 weeks of age.

Investigations were made of blood hemoglobin, serum protein, and incorporation of ^{14}C-DL-leucine into serum protein, and nitrogen balance after 8 weeks of treatment and before the 'switches' in the diet were made. These investigations were repeated after a further period of 8 weeks.

The data on growth are presented in Tables 42-45 and Fig. 3. As expected, weight gains during phase I (3-11 weeks of age) were least in the animals fed 5% protein and greatest in those fed 20%. Comparisons of males and females fed the 5%, 10% and 20% protein diet throughout showed some interesting features. Males gained more weight than females when fed the 10% and 20% protein diets but not when fed the 5% protein diet, suggesting that sex differences in growth are minimized or abolished when nutritional constraints affect growth.

In both males and females, the rate of body weight gain was maximum soon after weaning. The rate declined more abruptly in the 20% protein animals compared to the 10% and 5% animals. In the 20% protein males the rate of growth

Table 42 : Growth in phase I (3-11 weeks) of animals fed different levels of protein.

% protein in diet during phase I	Initial weight	at the end of phase I			
		Body weight (g)	Weight gain (g) per week	Weight gain as % control	% increment over initial weight
		mean ± se			
5	M (6)	63 ± 3[a]	2.5 ± 0.4[b]	11	51
	F (6)	67 ± 6[f]	3.3 ± 0.6[c]	20	60
	T (12)	65 ± 5	2.9 ± 0.3	16	56
10	M (3)	175 ± 6[a]	17.0 ± 0.8[b]	72	324
	F (3)	147 ± 5[f]	13.5 ± 0.6[c]	82	270
	T (6)	161 ± 7	15.3 ± 1.3	77	297
20	M (6)	232 ± 1[a]	23.8 ± 0.4[b]	100	442
	F (5)	172 ± 4[f]	16.4 ± 0.4[c]	100	312
	T (11)	202 ± 7	20.1 ± 0.8	100	377

a. Number in parentheses indicate no. of animals.

M - Male

F - Female

T - Total (M + F)

Values marked with the same letter are significantly different from each other at p less than 0.05.

Table 43 : Growth in phase II (11-20 weeks) of animals fed different levels of protein.

% protein in diet during phase		Initial weight	values at the end of phase II			
I	II		Body weight (g)	Weight gain (g) per week	Weight gain as % control	% increment over initial weight
			mean ± se			
5	5	M (3)	100±1^{b}	4.2±0.3^{a}	16	62
		F (3)	103±4^{g}	5.0±0.5^{j}	43	76
		T (6)	102±2	4.6 ±0.4	30	69
5	10	M (3)	235±5^{bc}	19.0±0.6^{ac}	87	271
		F (3)	204±5^{gh}	14.3±0.5^{jk}	124	164
		T (6)	220±7	16.7±0.9	106	218
10	10	M (3)	278±11^{c}	11.4±0.5^{e}	57	59
		F (3)	230±4^{h}	9.1±0.5^{k}	78	56
		T (6)	254±13	10.3±0.7	68	58
20	10	M (3)	362±9^{d}	13.8±0.5^{f}	63	52
		F (2)	258±4^{i}	10.2	88	55
		T (5)	310±13	12.0±1.1	76	54
20	20	M (3)	452±4^{d}	27.8±0.3^{f}	100	98
		F (3)	280±6^{i}	11.6±0.1	100	59
		T (6)	366±12	19.7±1.8	100	79

Numbers in parentheses indicate no. of animals.

M - Male

F - Female

T - (Total M + F)

Values marked with the same aletter are significantly different from each other at p less than 0.05.

Phase I - 3-11 weeks.

Phase II - 11-20 weeks.

Table 44 : Growth in phase III (20-23 weeks) of animals fed different levels of protein.

% protein in diet during phase			Initial weight	values at the end of phase III			
I	II	III		Body weight (g)	Weight gain (g) per week	Weight gain as % control	% increment over initial weight
				mean ± se			
5	5	5	M (3)	106±1	2.0±0.3	19	6
			F (3)	110±3	2.3±2.0	32	7
			T (6)	108±2	2.2±0.7	26	7
5	10	10	M (3)	307±7	24.0±3.3	236	33
			F (3)	229±5	8.3±3.0	115	13
			T (6)	268±12	16.0±10	176	23
10	10	10	M (2)	343	24.3	24	27
			F (2)	239	3.3	46	4
			T (4)	291±30	13.8±9	35	16
10	10	5	M (1)	297	0.6	7	1
			F (1)	228	-10	-14	-
			T (2)	263			
20	10	10	M (3)	414±2[e]	17.3±2.7	17	14
			F (2)	266	3.0	41	3
			T (5)	340±37	10.2±4.1	29	9
20	20	20	M (3)	483±1[e]	10.3±0.7	100	7
			F (3)	302±5	7.3±1.3	100	8
			T (6)	393±52	9.0±0.6	100	8

Number in parentheses indicate no. of animals.

M - Male; F - Female; T - Total (M + F).

Values marked with the same letter are significantly different from each other at p less than 0.05.

Phase -I 3-11 weeks.
Phase -II - 11-20 weeks.
Phase III - 20.23 weeks.

Table 45 : Growth in phase IV (23-31 weeks) of animals fed different levels ofprotein.

% protein in diet during phase				Initial weight	Values at the end of phase IV			
I	II	III	IV		Body weight (g)	Weight gain (g) per week	Weight gain as % control	% increment over initial weight
1	2	3	4	5	6	7	7	9
						mean ± se		
5	5	5	5	M (3)	124 ± 6	2.0 ± 0.5	178	15
				F (3)	128 ± 6	2.5 ± 0.8	125	18
				T (6)	126 ± 3	2.3 ± 0.6	152	17
5	5	5	10	M (2)	435	22.5	1111	32
				F (2)	259	3.3	163	12
				T (4)	347 ± 49	12.9 ± 6.7	637	22
5	10	10	5	M (1)	336	3.9	344	10
				F (1)	258	2.9	144	10
				T (2)	297	3.4	244	10
10	10	10	10	M (2)	442	8.8	788	20
				F (2)	278	4.5	225	15
				T (4)	360 ± 51	6.7 ± 1.5	507	18
10	10	5	5	M (1)	369	8.8	767	20
				F (1)	277	5.5	339	19
				F (2)	323	7.2	553	20

contd...

Table 45 : contd.

1	2	3	4	5	6	7	8	9
20	10	10	10	M (3)	487 ± 9	6.3 ± 0.9	556	12
				F (2)	290	2.5	125	8
				T (5)	389 ± 54	4.4 ± 1.2	341	10
20	20	20	20	M (3)	499 ± 6	1.1 ± 0.4	100	2
				F (3)	321 ± 5	2.0 ± 0.3	100	5
				T (6)	410 ± 48	1.6 ± 0.3	100	4

Numbers in parentheses indicate no. of animals.

M - Male

F - Female

T - Total (M + F)

Phase - I - 3 - 11 weeks.

Phase - II - 11 - 20 weeks.

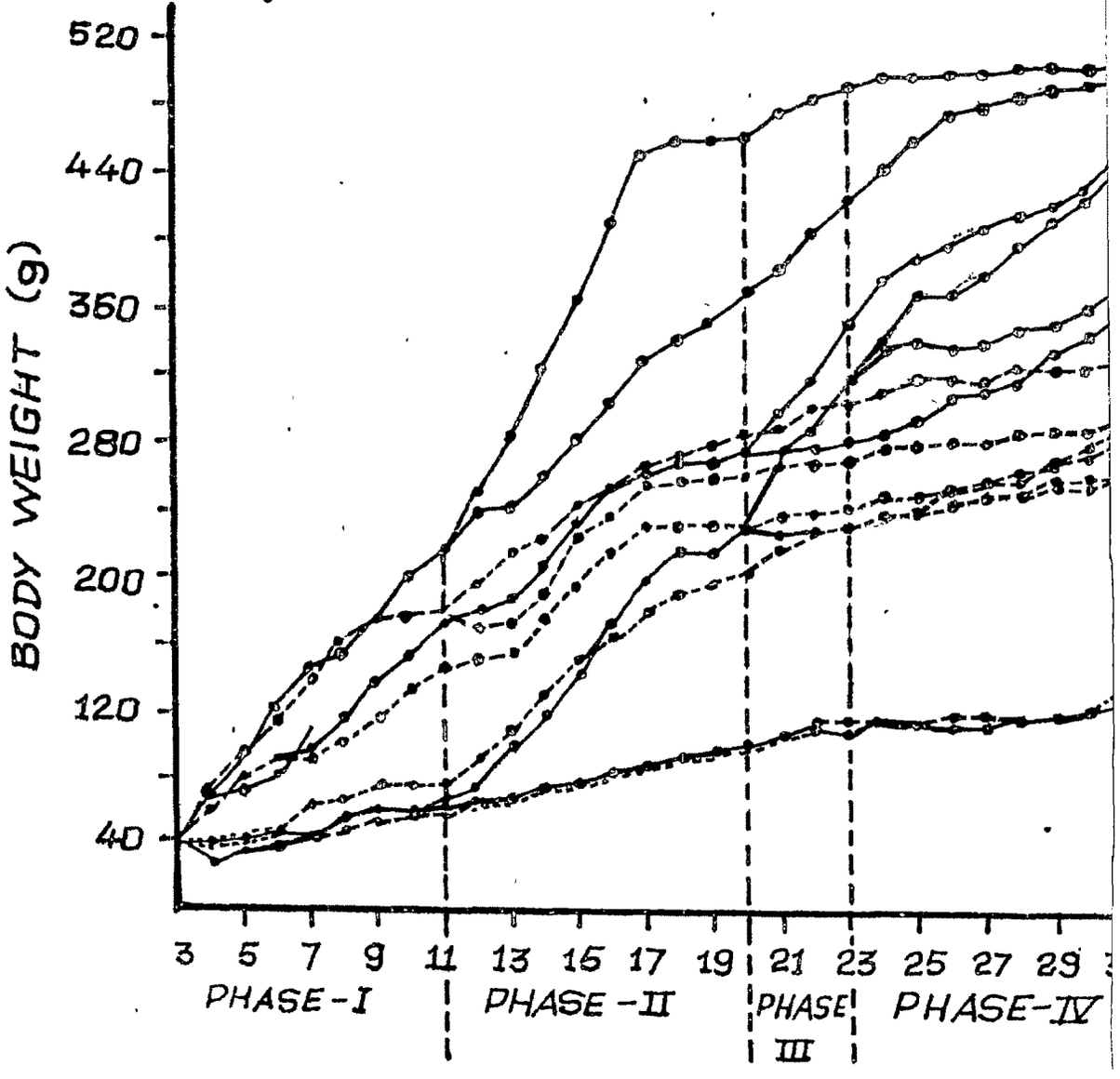
Phase - III - 20 - 23 weeks.

Phase - IV - 23 - 32 weeks.

FIG: 3

GROWTH CURVES OF RATS SUBJECTED TO VARIA
IN DIETARY PROTEIN CONTENT & TO CHANGES
THE SAME.

I M, I F } 5% → 10% PROTEIN DIET
II M, II F } 10% PROTEIN DIET
III M, III F } 20% PROTEIN DIET
IV M, IV F } 20% → 10% PROTEIN DIET
V M, V F } 5% — 10% → 5% PROTEIN DIET
VI M, VI F } 10% → 5% PROTEIN DIET
VII M, VII } 5% PROT



declined markedly after 15-20 weeks of age. The decline was more gradual in females (Fig. 3).

The differences between the 10% and 20% protein groups increased in males but became less in females. This may be due to the steeper growth curve of the males as compared to the females. For presumably the same reason, in the groups fed 20% protein diet, sex differences became larger during 11-20 weeks.

In animals fed 10% protein, the relatively more rapid growth phase was over a longer period with the growth deficits getting less with the progress of time.

The switch from 5% to 10% protein diets resulted in catch-up growth in the case of males as well as females, as judged by growth rates.

As can be seen from Fig. 3, the catch-up growth continued till 31 weeks of age in the case of males but slowed down considerably at 17 weeks of age in the case of x females.

The switch from a 20% to a 10% protein diet resulted in deceleration of growth only in the case of males. However, even in this case, growth was initially greater (Fig. 3) than in the animals fed 10% even though the growth rates for the 11-20 week period were comparable (Table 43) suggesting the possible operation of carry over effects from the previous diet. But this was not the case in females. Clearly, the

adaptation to change to a high protein diet from a low protein diet seem rapid and prolonged, while the effects of switches from high to low protein diets are not well defined.

Originally, the experiment was designed only to investigate the effects of switches from a 5% or 20% diet to a 10% diet. Because of the interesting observations reported above, some more switches were made. Thisresulted ina small sample size at this point and any observation on the changes made must be considered of a preliminary nature.

The switch from a 10% to a 5% protein diet was associated with a dramatic decline in weight gain with adaptation to the change after 3 weeks. This observation prompted another switch, that of the 5% → 10% animal back to a 5% proteindiet. When this was done, there was an abrupt decline in body weight gain in the case of the males but not in the case of the females (Table 45, Fog. 3). The most remarkable observations were however, the generally greater gains of all the groups fed less than 20% protein as compared to this group. This suggests a gradual adaptation to the deficient diet and extension of the growth phase. Similar observations have been made with regard to the proliferation phase of the cell cycle even in tissues such as nervous tissue (Lewis et al, 1975). Parallel studies in this laboratory have shown the greater growth span of undernourished adolescents (Jyoti, 1982).

The observations are of relevance for the situation in which children with severe protein-calorie malnutrition are given an improved diet in the hospital or in a nutrition rehabilitation centre and sent back home where in all probability they may continue to get a poor diet. In parallel studies in this laboratory in both situations their growth rates are found to be higher than in controls (Rajalakshmi and Ramtapkumar, unpublished).

Data on blood hemoglobin and serum protein are presented in Table 46 and individual values in Table 47. The differences between the groups were as expected during phase I. It appears that a 10% protein diet may be adequate to maintain blood hemoglobin and serumprotein levels at levels not significantly different from levels found in 20% protein animals.

In this connection, proteindeficiency is found to affect hemoglobin synthesis because of its effect on iron utilisation and absorption (Layrisse and Martinez-Torres, 1971; El-Schobaki et al, 1972) mediated by its effect on transferrin synthesis (Morgan and Peter, 1977) as also the synthesis of hemoglobin itself (Weech et al, 1937). However, theeffects of protein defiebency on blood hemoglobin levels seem to be variable as is suggested by ppdvious studies in this laboratory where protein deficiency did not always have theexpected effect on blood hemoglobin of children suffering from severe protein-calorie malnutrition. While the average blood hemoglobin level in such children is of the order of 7-8g per 100 ml as compared to 11-12g per 100 ml in controls, individual children

Table 46 : Effect of feeding different levels of protein on blood hemoglobin and serum protein.

% protein in diet during weeks		Hemoglobin (g per 100 ml) at the end of week		Serum protein (g per 100 ml) at the end of week	
3 - 11	11 - 20	11	20	11	20
		mean ± s.e.**			
5	5	6.6[a] ± 0.2	8.6[bc] ± 0.2	5.73[ed] ± 0.14	5.72[f] ± 0.11
5	10	8.8 ± 0.3	9.7 ± 0.2	5.88 ± 0.12	6.15[fg] ± 0.11
10	10	10.7 ± 0.3	11.0 ± 0.3	6.53[e] ± 0.10	6.53[g] ±0.09
20	10	11.9[a] ± 0.2	11.7[c] ± 0.2	6.65[d] ± 0.05	6.62 ± 0.07
20	20	12.0 ± 0.2	12.0[b] ± 0.2	6.67 ± 0.06	6.68 ± 0.05
		Values as % control values			
5	5	72	72	86	86
5	10	73	81	88	92
10	10	89	92	98	98
20	10	99	98	100	99
20	20	100	100	100	100

** based on 6 observations per group.

Values marked with the same letter are significantly different from each other at p lessthan 0.05.

Table 47 : Longitudinal data on blood hemoglobin and serum protein changes of animals fed different levels of protein.

% protein in diet during weeks		Animal No.	Blood hemoglobin (g/100 ml) at the end of week		Serum protein (g/100 ml) at the end of week	
3-11	11-20		11	20	11	20
1	2	3	4	5	6	7
5	5	1 M	8.3	8.3	5.5	5.5
		2 M	9.5	9.3	6.3	6.0
		3 M	8.8	8.9	5.8	6.0
		4 F	7.9	8.3	5.5	5.5
		5 F	8.5	8.2	5.6	5.7
		6 F	8.7	8.8	5.7	5.6
		Mean	8.6	8.6	5.73	5.72
		± se	± 0.2	± 0.2	±0.14	±0.11
5	10	7 M	8.0	9.0	5.5	5.5
		8 M	9.1	9.5	6.3	6.0
		11 M	9.2	9.6	5.8	6.0
		9 F	8.6	10.0	5.5	5.5
		10 F	9.0	10.2	5.7	5.6
		12 F				
		mean	8.8	9.7	5.73	5.72
		± se	± 0.3	± 0.2	±0.14	±0.11
10	10	13 M	10.5	10.5	6.4	6.4
		14 M	11.3	11.2	6.2	6.3
		17 M	11.4	11.5	6.5	6.4
		15 F	11.9	12.1	6.8	6.7
		16 F	10.2	10.5	6.6	6.7
		18 F	10.0	10.4	6.7	6.7
		Mean	10.7	11.0	6.53	6.53
		± se	± 0.3	± 0.3	±0.10	±0.09

contd..

Table 47 : contd.

1	2	3	4	5	6	7
20	10	19 M	12.1	12.2	6.6	6.5
		20 M	11.3	11.0	6.8	6.8
		21 M	11.9	11.5	6.7	6.6
		22 F	11.8	11.5	6.7	6.7
		23 F	12.3	12.2	6.6	6.7
		Meankk.	11.9	11.7	6.65	6.62
		± se	± 0.2	± 0.2	±0.05	±0.07
20	20	24 M	12.5	12.5	6.5	6.6
		25 M	11.9	11.8	6.6	6.7
		26 M	12.1	12.2	6.8	6.9
		27 F	11.5	11.3	6.7	6.6
		28 F	11.8	11.9	6.8	6.7
		29 F	12.1	12.4	6.6	6.6
		Mean	12.0	12.0	6.67	6.68
		± se	± 0.2	± 0.2	±0.06	±0.05

are found to have both higher values of the order of 12g, and lower values of the order of 3g per 100 ml. Occasional cases show normal levels of hemoglobin with low (< 5 g/100 ml)levels of serum protein (ONR Reports, 1972-75; Pratapkumar, 1982).

Thus, in protein deficiency, a fall in both hemoglobin and serum protein as well as in other constituents such as serum transferrin and liver protein is expected (cf. Vulterinova, 1981) but the relative decreases seem to vary with the experimental conditions as is seen in various animal studies conducted in this laboratory (Majumdar, 1965; Tambe, 1967; Chari, 1967; Khanum,1967) and perhaps with the stores of iron acquired before depletion.

Changes from a 5% or 20% protein diet to a 10% protein diet had the expected effect on both blood hemoglobin and serum protein but theeffects were much less dramatic than in the case of weight gain. In this connection, both hemoglobin and serum protein are found to show only a slow response to nutritional rehabilitation in malnourished children, even though the catch-up growth is very rapid (Pratapkumar,1982). In fact, changes in serum abluminlevels have been considered sensitive indices of the extent of recovery from protein malnutrition (Thomas and Comb, 1967). In these children, blood hemoglobin shows a much slower response possibly because of the involvement of other factors such as iron and vitamin C deficiencies or because restoring serum albumin to normal levels has higher metabolic priority.It is known that a rise in blood hemoglobin is found only after iron stores are replenished.

The data on nitrogen balance studies are presented in Tables 48-50. Eight weeks after feeding of 5,10 or 20% protein diets to weanling animals, neither apparent digestibility nor per cent absorbed nitrogen retained varied with the amount of dietary protein. Differences in the amount of nitrogen retained appear to be associated wnly with differences in intake, although the low fecal and urine excretions of N suggest economy in utilization of protein. However, nitrogen retention increased markedly in the 5% group during phase II, suggesting the evolution of adaptive mechanisms. The improved retention is also reflected in the improved weight gain during this period (Table 43). This improvement is all the more remarkable as all the other groups showed a reduction in the percentage of absorbed N retained, an observation consistent with the fact that the biological value of proteins declines with age. In spite of this, the group switched from 5% to 10% showed an improved weight gain during this phase, consistent with thegreater amount of nitrogen retained in absolute terms.

The increased retention of nitrogen by the 5% protein animals during phase II contrasts with the decreased retention in the case of the20% animals in both relative and absolute terms. This is consistent with the acceleration of growth in the former and deceleration in the latter.

Nitrogen balance data are held to be suspect because of the built-in errors in the same due to over-estimation of food intake and underestimation of urine and fecal losses by other

Table 48 : Food, fecal and urine nitrogen of animals fed different levels of protein.

% protein diet during phase			Food nitrogen (mg)		Fecal nitrogen (mg)		Urine nitrogen (mg)	
I	II		I	II	I	II	I	II
			mean ± se					
5	5	M (3)	131±12	189±19	15±1	19±1	28±4	15±1
		F (3)	88±16	233±17	17±5	19±3	17±3	18 ±8
		T (6)	109±15	208±15	16±2	19±2	23±4	16±4
5	10	M (3)	117±15	670±24	22±3	65±6	31±4	243±19
		F (3)	84±9	559±29	13±2	46 ±6	24±2	194±13
		T (6)	101±12	614±23	17±3	56±8	28±4	219±21
10	10	M (3)	810±32	1087±26	84±6	105 ±8	189±25	437±28
		F (3)	685±68	901±5	53±3	79±2	107±11	400±37
		T (6)	723±57	994±48	69±9	92±1	148±24	419±24
20	10	M (3)	1606±54	1041±32	149±17	101±16	325±47	412±27
		F (2)	1022±132	934±44	122±32	91±2	200±15	417±98
		T (5)	1423±131	988±38	138±15	96±8	263±39	415±50
20	20	M (3)	1699±72	1687± 34	168±13	161±8	299±22	534±37
		F (3)	103±175	1267±157	121±5	120±10	165±61	543±124
		T (6)	1531±135	1477±131	145±4	141±12	232±29	493±53

Numbers in parentheses indicate no. of animals.

M - Male, F - Female, T - Total (M + F).

Phase I - 3-11 weeks.

Phase II - 11-20 weeks.

Table 40 : Apparent digestibility, mg N retained and per cent absorbed N retained in animals fed different levels of protein.

% protein in diet during phase			Apparent digestibility		mg N retained		per cent absorbed N retained	
I	II		I	II	I	II	I	II
					mean ± se			
5	5	M (3)	89,3±0.3	90.2±0.9	87±7	153±17	75.7±1.8	91.0±0.9
		F (3)	86.0 (80,92)	91.3±1.2	54±13	190± 5	74.0±6.0	91.7±3.4
		T (6)	87.7±3.0	90.7±0.9	68±11	172±13	74.7±3.0	91.4±1.7
5	10	M (3)	89.7±5.0	91.3±0.9	64±12	361±27	76.7±2.1	69.7±3.3
		F (3)	84.0±1.6	91.7±0.7	47± 5	320±13	75.7±0.3	62.0±0.6
		T (6)	87.4±3.0	91.0±0.8	56±14	342± 9	76.2±1.0	60.8±1.7
10	10	M (3)	89.7±1.2	90.2±0.3	537±25	548±28	74.0±2.7	55.7±2.8
		F (3)	91.7±1.5	91.3±0.3	475±59	456±21	81.3±0.3	51.3±4.7
		T (6)	90.7±1.1	90.8±0.3	506±29	502±29	77.7±2.2	55.5±2.8
20	10	M(3)	91.1±0.7	90.4±1.4	1132±70	565±35	77.7±3.6	56.3±3.9
		F (2)	89.5 (88,91)	89.3±0.3	822±45	426±55	80.0±4.2	52.0±8.3
		T (6)	90.3±0.7	899 ±0.7	1008±97	495±47	78.6±2.5	54.2±4.5
20	20	M (3)	90.2±1.2	90.3±0.7	1232±66	992± 6	80.7±0.9	65.0±1.6
		F (3)	88.7±1.9	90.3±0.9	847±113	693±28	81.0±1.4	61.7±5.6
		T (6)	89.5±1.1	90.3±0.6	1115±97	843±76	80.8±0.6	63.4±2.9

Numbers in parentheses indicatd no. of animals.
M - Male, F - Female, T - Total (M + F).
Phase - I - 3-11 weeks.
Phase - II - 11-20 weeks.

Table 50 : Nitrogen balance data; values as per cent reference values* for animals fed different levels of protein.

% protein in diet during phase			Food N		Fecal N		Urine N		Apparent digestibility		mg N retained		% per cent absorbed N retained	
I	II		I	II	I	II	I	II	I	II	I	II	I	II
							values as % reference value*							
5	5	M	8	11	9	12	9	8	99	100	7	15	94	140
		F	8	18	14	16	10	4	97	101	6	27	91	149
		T	17	14	11	13	9	3	98	101	6	20	83	144
5	10	M	7	40	13	40	10	46	99	101	5	36	95	92
		F	7	44	11	38	15	43	95	102	6	46	93	101
		T	7	42	12	39	13	44	97	101	5	41	94	96
10	10	M	48	64	50	65	63	82	99	100	44	55	92	96
		F	56	71	44	66	65	88	103	101	56	66	100	83
		T	52	67	47	65	63	85	101	100	45	60	96	85
20	10	M	95	62	89	63	109	77	101	100	92	57	96	87
		F	90	74	101	76	121	92	101	99	97	62	99	84
		T	93	67	95	68	115	84	101	100	90	59	97	86

* the group fed 20% protein throughout.

routes (Hegsted, 1978). However, in the present context where different groups are compared, they are expected to be of reasonable validity. The consistency of the values obtained for phase I and II for apparent digestibility suggest the reliability of the food and fecal estimates. The comparability of the values for percent absorbed nitrogen retained also reinforces this impression. Again, the amounts of N retained by the 10% animals during phase I and II are nearly the same, suggesting that the differences in the other cases are reasonably valid for comparative purposes.

Results presented in Tables 51-54 on incorporation of C-14-DL-leucine into serum protein show that at the end of phase I, the label incorporated was directly related to the amount of protein in the diet, being maximum in the 20% protein diet. The label was injected on the basis of body weight and the counts calculated per g serum protein and it is hoped that this procedure would help eliminate differences in body size and serum protein levels. It would therefore be reasonable to attribute the differences in radioactivity to either differences in synthetic ability or in catabolic rate.

The following observations emerge from a scrutiny of the data :

The amount of label varied with the amount of dietary protein, suggesting differences in either rates of synthesis or catabolism. The fact that these differences are more marked in the

Table 51 : Incorporation of C^{14}-DL-leucine into serum protein in rats fed different levels of protein.

I. At the end of phase - I

% protein in duet during Phase - I	Hours after injection							After 10 weeks
	12	18	24	36	48	72	112	
	cpm/g serum protein - mean ± s.e.*							
5	1361^{a} ± 49	1297^{b} ±125	1185^{c} ± 50	997^{e} ±114	708^{g} ± 76	512 ± 54	x 317 ±36	249^{hi} ±36
10	1571^{a} ± 49	1577^{b} ± 46	1400^{c} ± 44	1140^{d} ± 24	899^{f} ± 31	574 ± 41	313 ± 28	113^{i} ± 36
20	2044^{a} ±139	1990^{b} ±134	1702^{c} ± 32	1511^{de} ± 22	1234^{fg} ± 74	606 ± 28	420 ± 32	103^{h} ± 20

II. At the end of Phase - II

% protein in diet during phase		Hours after injection				
I	II	0 **	3	6	12	24
		cpm/g serum protein - mean ± s.e.*				
5	5	249 ± 36^{d}	1020 ± 63	1427 ± 189^{dg}	1352 ± 78^{jk}	979 ± 75^{psv}
5	10	162 ± 23	1107 ± 55	1401 ± 61^{eh}	1516 ± 63^{lmn}	1181 ± 76^{qt}
10	10	113 ± 36	1205 ± 38	1412 ± 26^{ai}	1655 ± 68^{bo}	1422 ± 108^{cuv}
20	10	61 ± 20	1069 ± 72	1905 ± 71^{ae}	2223 ± 54^{bjlm}	1781 ± 92^{cpq}
20	20	103 ± 20^{d}	1020 ± 51	1990 ± 53^{ghi}	2105 ± 77^{kno}	1814 ± 71^{stu}

* based on 6 observations per group.
Values marked with the s ame letter are significantly different s from each other at $p < 0.05$.
** residu$al activity from the close administered during phase I.

Table 52 : Incorporation of C^{14}-DL-leucine into serum protein in rats fed different levels of protein- values expressed as percent 12 hr. values.

I. At the end of phase - I

% protein in diet during phase - I	Hours after injection							
	14	18	24	36	48	72	112	10 weeks
5	100	95	81	74	52	38	23	18
10	100	100	89	73	57	37	20	7
20	100	98	83	74	60	30	21	5

II. At the end of Phase - II

% protein in diet during phase		Hours after injection			
I	II	3	6	12	24
5	5	75	105	100	72
5	10	73	92	100	77
10	10	73	85	100	86
20	10	48	86	100	80
20	20	48	95	100	86

Table 53 : Incorporation of C^{14}-DL-leucine into serum protein in rats fed different levels of protein - phase I and phase II values compared.

Hour after injection	Phase	Group				
		5 → 5	5 → 10	10 → 10	10 → 20	20 → 20
		mean ± s.e.*				
12	I	1381 ± 18	1341 ± 79	1571 ± 49	2019 ± 163	2069 ± 114
	II	1352 ± 72	1516 ± 63	1655 ± 68	2223 ± 54	2105 ± 77
24	I	1192 ± 23[a]	1178 ± 76	1400 ± 44	1663 ± 56	1741 ± 7
	II	979 ± 75[a]	1181 ± 76	1422 ± 108	1781 ± 92	1814 ± 71

* Based on 6 observations per group.

Values marked with the same letter are significantly different from each other at $p < 0.05$.

Table 54 : Incorporation of C^{14}-DL-leucine into serum-protein in rats fed different levels of protein - values expressed as percent control values.

I. At the end of Phase - I

% protein in diet during Phase -I	Hours after injection						
	12	18	24	36	48	72	112
5	67	66	69	65	56	78	86
10	75	81	80	74	69	87	85
20	100	100	100	100	100	100	100

II. At the end of Phase - II

% protein in diet during phase		Hours after injection				
I	II	0	3	6	12	24
5	5	240	100	72	64	54
5	10	157	109	70	72	65
10	10	110	118	71	89	78
20	10	59	105	96	106	98
20	20	100	100	100	100	100

earlier stage than in the later stages suggests that the initial differences are mainly due to differences in therates of synthesis.

The values at 12 and 72 h, on the other hand suggest a more rapid loss of label in the 20% group as compared to the other two groups. This impression is confirmed by the residual activity detected 10 weeks after the administration of the label.

In the above studies, radioactivity was monitored at 12 hours or more, The same was found to be maximum at 12 h which was also associated with the greatest differences in radioactivity between groups. If these differences are due to differences in turnover rates, monitoring the label at closer intervals should give a clearer picture. In the next series of investigations carried out at the end of phase II, therefore, the label was monitored at 3,6,12 and 24h following the administration of the label.

Again, peak values were reached only at 12 h in all the groups. The differences at this point and at 24 h compared very well in the two experiments. But the increase to peak values was more rapid in the 5% group contrary to expectation. This might be either because of differences in catabolic rates or because the 5% ✱ animals were in their extended growth phase during this period whereas inthe other groups growth had slowed down.

Of particular interest are the data on animalsswitched from 5% or 20% to 10% protein diets. It is interesting to note that in spite of the gross differences in weight gain, the data on radioactivity suggest a pattern resembling that in animals continued on these diets rather than that in animals fed the 10% diet from start. This suggests that turnover rates may be influenced appreciably by the diets to which the animals are first adapted. If this be the case, it would account for the differential effects on growth of a switch from a 5% or 20% to a 10% protein diet. The low plane of metabolism in the former would facilitate a better utilization of protein for growth ~~in the former case~~ whereas the reverse would be true of the latter.

A gradual adaptation to low protein diets in both cases is suggested by other studies on experimental animals (Jackson, 1937; Khan and Bender, 1974) and man (Darby et al, 1953; Murthy et al, 1955). Jackson (1937) reported that the amount of protein required to maintain constant body weight in young rats for 15 weeks decreased by about 13% after the first 3 weeks of feeding. The authors of the Vanderbilt study (Darby et al, 1953) as also Murthy and associates (1955) were mystified by their inability to find objective signs of protein deficiency in populations on low intakes of protein. This may well have resulted from a prolonged period of adaptation in the poor diet. A slow and steady growth rate leading to near

normal adult stature by six months of age is observed in weanling rats on 8% protein diets (Turner, 1973), a pattern similar to that in the 10% group in the present studies.

An increased efficiency of dietary protein utilization judged by nitrogen balance has been found in animals fed suboptimal quantity or poor quality protein during the post-weaning period alone or coupled with neonatal deprivation induced by feeding the mother low protein diets. This improvement was limited to the period of rehabilitation and ceased to exist once catch up was achieved (Barnes, et al, 1973; Barnes and Kwong, 1977). Similarly, Lang (1977) reported that in the malnourished child, repletion with maintenance levels of protein and calories produced an increased level of nitrogen retention, whereas similar levels inthe recovered child were less efficient. However, these studies were not concerned with the matabolic response of animals shifted from high to low protein diets although Allison(1950) observes in this connection that it requires more nitrogen to maintain equilibrium in an animal well fed with regard to protein than in one which is depleted.

In neither the present study nor the earlier study of Barnes and associates (1973) were differences in endogenous nitrogen losses that might have contributed to the apparent differences in efficiency of nitrogen utilization taken into account. However, such losses were determined in a subsequent

study by Barnes and Kwong (1977) over a 11 day period during which the animals were fed a protein free diet. The pattern of the results obtained were not altered when corrections for endogenous losses were applied making possible calculations for both, growth and maintenance. These findings confer greater validity on the present investigations, although, as pointed out by Hegsted (1976), data on endogenous nitrogen losses should be viewed with caution and scepticism because even small differences in excretion at zero intakes have large effects on calculated efficiency of utilization.

The liver has a key role in the adaptive processes because it is the site of urea formation from amino acids. In the liver, the proportion of conversion of circulating nitrogen to urea and retention within the amino acid pool for synthetic processes in various tissues depends on the plane of nutrition of the organism. As an immediate consequence of dearth, the synthetic rate of body protein goes down. The catabolic rate decreases more slowly (McFarlane, 1963; Garlick et al, 1975; Waterloo et al, 1977; Golden et al, 1977). Thus, for example, some reduction in serumprotein level is found before the levels become more or less steady (McFarlane, 1963). In the studies of Golden et al (1977) on severely malnourished children giving N-15 -glycine and monitoring the label in urinary urea, a significant correlation was found between protein flux and protein synthesis and the ad lib

dietary intake, nitrogen balance and weight change in children who were recovering or had recovered from severe PCM. Over the range of dietary intake of 60 to 270 cal/kg/day, protein synthetic rate was increased 5 fold but decreases in protein breakdown were small and did not contribute to changes in N balance or body weight.

In the present study also, a decreased incorporation of label in the chronically deprived group as well as some decrease in the rate of loss of label as judged from residual radioactivity in serum 10 weeks after injection was found. Previous studies report a decrease in uptake of label (Simm et al, 1975) or an increased retention(Narasinga Rao and Radhakrishnan,1966; Nettleton and Hegsted, 1974(a),(b); Gota and Kametaka, 1974; Milenkovic et al, 1974; NIN Annual Report, 1976) in liver of the protein and/or calorie deficient animal.

A lot of controversy exists with regard to the methodology and interpretation of studies on incorporation of label into plasma or serum protein. Some of the major objections are detailed below :

(1) Studies with reutilizable amino acids : In the adult man, the total body protein turnover per day is of the order of 300 g (San Pietro and Rittenberg, 1953) while the intake is about 50g. 250 g of body protein is made from amino acids which have been released into the metabolic

pool during the turnover of tissue protein. In malnutrition degradation of protein to urea decreases resulting in increased reutilization. This is demonstrated by Dallman and Manies (1973) using two labels, viz. ^{14}C-guanidine arginine and ^{3}H-arginine. The former is reutilized, if at all, to a very small extent; the latter has high reutilization. Differences were seen in retention of the two labels. Results using the latter indicate a decreased rate of turnover, while use of the former indicate no change. Thus, the use of a reutilizable isotope can give no idea of the actual turnover rates of protein.

(2) Size of the metabolic pool : During protein depletion, the plasma protein specific activity or the percent of a given dose of labelled amino acid which appears in intravascular plasma protein is dramatically increased. This does not indicate a change in turnover rates because similar results are obtained, if the size of the metabolic pool is altered.

(3) Incorporation rates in tissues other than the test tissues : e.g. amino acids available for incorporation into plasma protein are affected by the turnover rates of other tissue proteins.

(4) It is assumed that the rate of incorporation of an amino acid into a specific protein directly reflects the rate of synthesis of that protein. However, the assumption is true only if the precursor specific activity is constant. In practice, the precursor specific activity following a single injection or feeding of an amino acid is far from constant (McFarlane, 1964; Jarnam, 1965). Hence, either specific precursor activity should be determined or it should be maintained constant. The latter is preferred. It is achieved by the constant infusion of label till the protein precursor reaches a more or less constant plateau level when rate of entry of label into the metabolic pool equals its rate of loss.

In the present study, a reutilizable amino acid (DL-leucine) is used as label and measures made after a single pulse without any idea of the precursor pool activity of the protein turnover rates in other tissues. However, information obtained by a comparison of the patterns in the various groups yields useful results and the consistency of this pattern in the two experiments seems to lend greater plausibility to the results obtained.

In conclusion, animals fed diets providing 5,10 and 20% protein were found to differ with regard to growth rate as may be expected, but growth deficits as per cent

of control values tended to decline with the progress of treatment suggesting the intervention of age and adaptive mechanisms as additional factors. The deficits were less in the case of females.

The differences in the growth rates of males and females in the 20% protein group were reduced in animals fed a 10% protein diet and abolished in those fed a 5% protein diet.

Animals switched from a 5% to a 10% protein diet showed a much more efficient and rapid adaptation to the switch than those switched from 20% to 10% protein diets. The two groups differed from each other and from those fed the 10% protein diet, with respect to body weight gain, nitrogen balance, blood hemoglobin, serum protein and incorporation of labelled leucine into serum protein.

The studies underline the complexities of adaptation mechanisms and the importance of previous dietary history in interpreting present responses in balance studies. They are of significance in the interpretation of balance studies carried out in human populations and would account for many of the anomalies found.

Experiment - 4

Response of rats fed on a wheat diet with and without addition of lysine at different ages as judged by growth, nutritional status and incorporation of a labelled amino acid in serum protein.

The studies just described and these carried out elsewhere show that the age of the animal is an important factor in the adaptation of the animal to a diet low in protein. That age is a critical factor in the utilization of proteins which are poor in quality is suggested by several studies (cf. Irwin and Hegsted, 1971). As is well known, protein requirements consist of the requirements for two separate components, namely, maintenance or, in other words replacement of losses resulting from tissue breakdown and growth. The two components differ with regard to their relative contributions at different ages. They also differ with regard to the adaptation mechanisms they can possibly invoke in the animal subjected to dietary deprivation. At least theoretically, some recycling of essential amino acids derived from tissue breakdown for their renewal is possible (Dallman and Manies,1973) whereas the requirement for tissue growth will have to be met from exogenous sources. On the other hand, we cannot make an arithmetic approach to requirements during growth which include requirements for maintenance growth as the requirement for

maintenance may be minimised during growth by the more efficient ~~consumption~~ conservation. In fact endogenous losses of N are found to be less during growth and pregnancy. In this connection, while foods of high protein quality, such as, for example albumin, yield comparable scores for BV in the case of young and old animals, foods of poor protein quality such as corn or wheat yield very different scores for animals and human subjects differing in age. for instance, the protein quality of wheat gluten was found to be 0.40 and 0.65 respectively for the growing and adult rat (Allison, 1964).

The assessment of protein quality has assumed importance because dietary protein allowances have no relevance unless the quality of dietary protein is taken into account. Several biological and chemical methods are available for the assessment of protein quality and the latter depend on the formulation of a standard reference protein of known amino acid composition In the former reference protein (FAO, 1957) no account was taken of age differences in amino acid requirements. This has been sought to be rectified in the revised allowances (FAO, 1973) which give different patterns for infants, children and adults, The most striking differences are to be found with respect to lysine which is an amino acid whose supply is critical in diets based on cereals.

The lysine content of the pattern recommended for school boys is higher than that for infants and for children (FAO,

1973). This recommendation is based on a Japanese study (Nakagawa et al,1961) in which the amounts of lysine used for determining amino acid requirements were 2.4, 1.6, 1.2, 0.6 and 0 g in a diet providing 12 g of nitrogen whereas more graded doses might have given a different picture. Similarly the criteria used for assessing amino acid adequacy also vary in different studies with regard to total amount of N, type of non-essential N, form of essential N, amountsof various amino acids added, period of study and criteria used (Rose, 1949, 1957; Holt et al, 1960; Leverton, 1959; Swendsdd et al, 1956; 1959) and discussed by Irwin and Hegsted (1971).

Lysine supplementation to cereals has been advocated (United Nations, 1967; FAO, 1965; Rosenberg et al, 1954) and implemented (Japan, 1963; Westerman et al, 1957(a), Bressani et al, 1958; Scrimshaw et al, 1958) in several studies.

Beneficial effects have been claimed for such supplementation inthe case of school boys (Kitajima, 1969) but other studies have failed to give convincing results (Gershoff et al, 1975, 1977; Gershoff, 1977). In studies in this laboratory a school lunch providing wheat and bengal gram in the ratio 4:1 was not found to be demonstrably better than one providing them in the ratio 8:1 in spite of their appreciable difference in lysine content (Sail, 1970).

Although an animal model may be hardly relevant for an elucidation of this problem, it was considered worthwhile to investigate the effects of lysine supplementation to wheat on the growth and/or biochemical status of animals at different ages. The ages chosen (3, 13, 26 and 52 weeks at start) were designed to correspond to periods of rapid growth, puberty and adulthood with slowed down growth and after cessation of growth. Weekly body weights were recorded and blood hemoglobin, serum protein, liver protein and rate of loss of incorporated label from serum protein were measured 8 weeks after the experiment was begun.

In the second part of the experiment, the effects of lysine supplementation following prolonged deprivation is studied. 3 week old mk male rats were fed either a wheat diet or the same supplemented with lysine for a period of 20 weeks.

The growth curves of the various groups are presented in Figs. 4,5 and data on growth abstracted in Table 55. It can be seen that the growth rate of the 3 week old wheat fed group was demonstrably inferior to that of the wheat + lysine fed group. Weight gain remained significantly less when 13 week old animals were deprived of lysine, though their body weights were not significantly different. However, at the later ages of 26 and 52 weeks, lysine deprivation had no apparent effect on growth. When the animals were fed wheat

FIG. 4

GROWTH OF RATS FED ON A WHEAT DIET WITH OR WITHOUT ADDITION OF LYSINE AT DIFFERENT AGES.

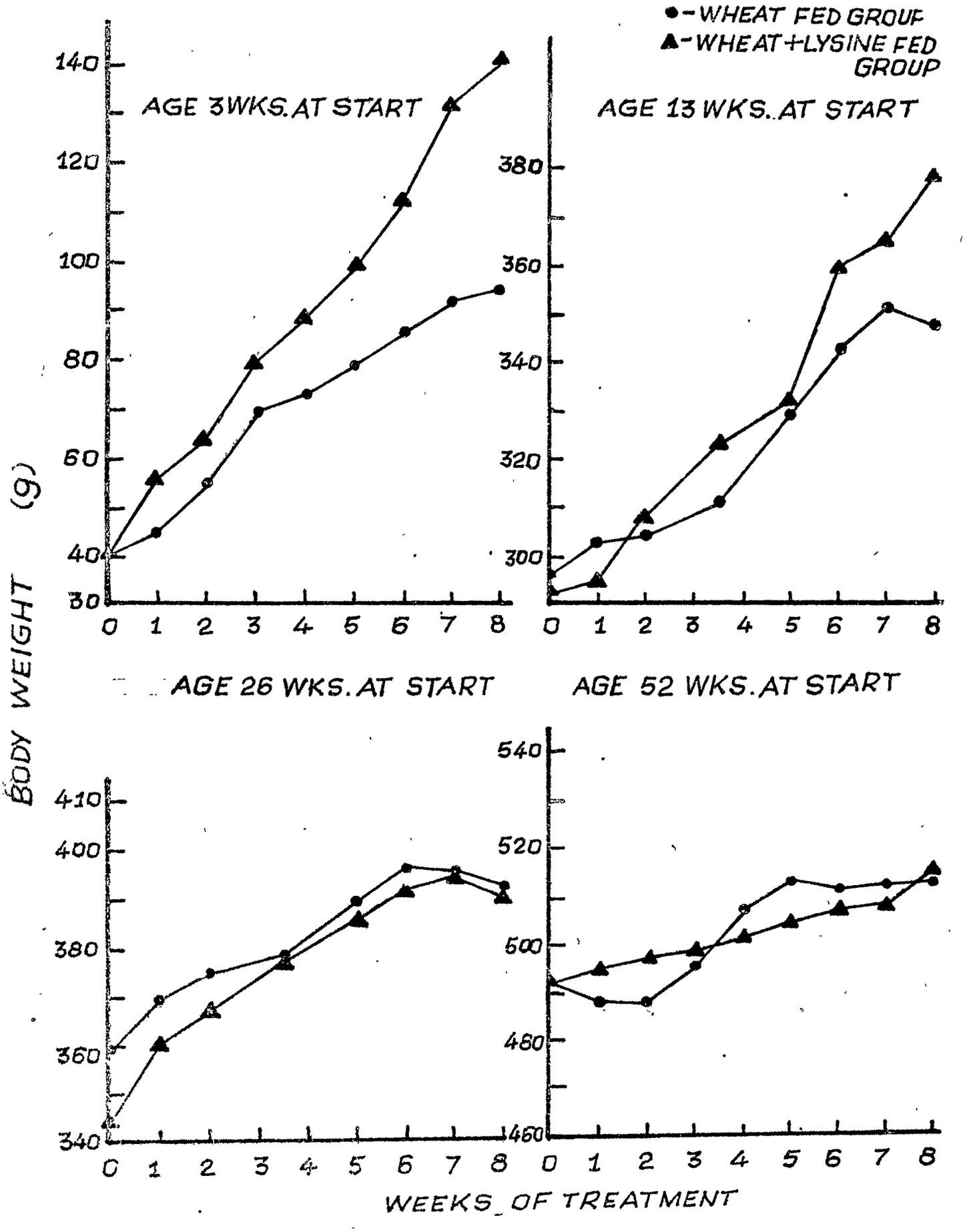


FIG.-5-GROWTH OF RATS FED ON A WHEAT DIET WITH & WITHOUT ADDITION OF LYSINE FROM WEANING ONWARDS.

I WHEAT DIET CONTD.
II WHEAT → WHEAT + LYSINE
III WHEAT + LYSINE → WHEAT
IV WHEAT + LYSINE (CONTD.)

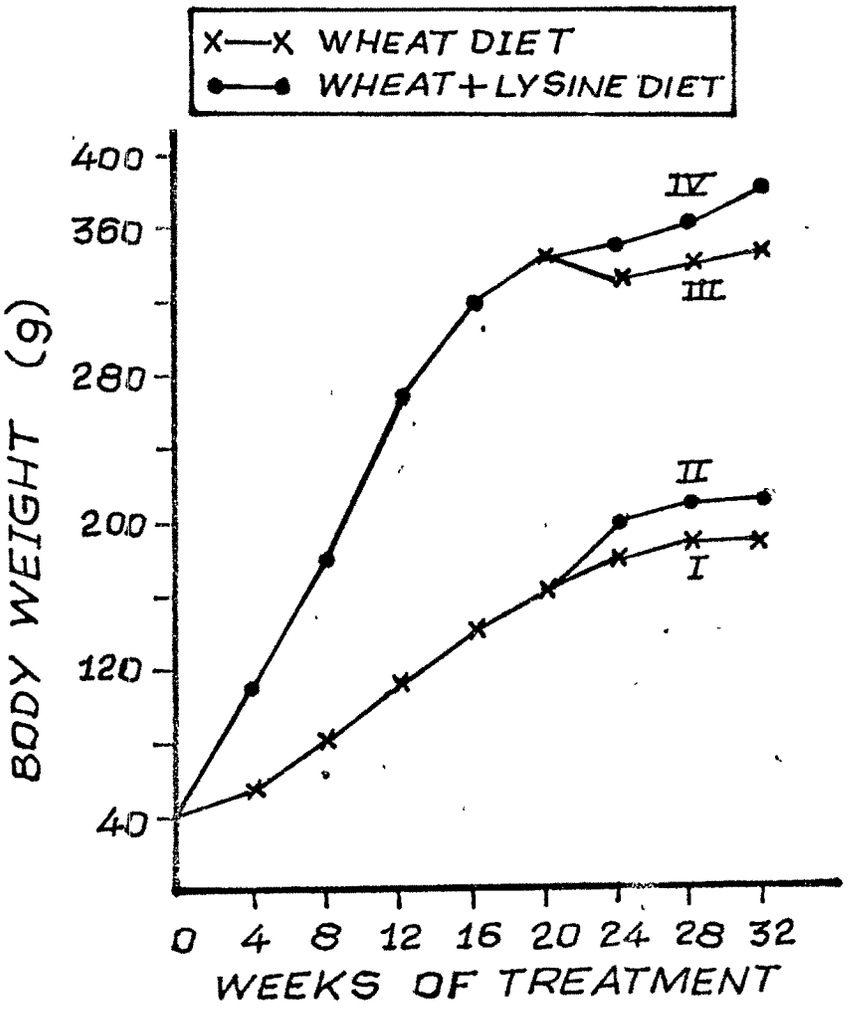


Table55 : Growth of rats fed wheat diet with and without addition of lysine at different ages.

Age (wks)		Diet	Body weight		Body weight gain	Weight gain per week
Initial	Final		Initial	Final		
			mean ± se[1]			
3	11	W	39 ± 1	95 ± 6[a]	56 ± 6[b]	7.0 ± 0.8[d]
		W + L	39 ± 1	142 ± 6[a]	103 ± 6[b]	12.9 ± 0.8[d]
13	21	W	297 ± 6	348 ± 9	47 ± 5[c]	5.9 ± 0.6[h]
		W + L	292 ± 5	378 ± 13	86 ± 11[c]	10.8 ± 1.4[h]
26	34	W	354 ± 8	392 ± 12	36 ± 6	4.6 ± 0.8
		W + L	343 ± 9	390 ± 13	59 ± 13	7.4 ± 1.6
52	60	W	492 ± 10	513 ± 12	28 ± 4	3.5 ± 0.5
		W + L	492 ± 7	515 ± 7	24 ± 4	3.0 ± 0.5
3	23	W	42 ± 3	168 ± 14[f]	126 ± 14[e]	6.3 ± 0.7[g]
		W + L	41 ± 1	345 ± 11[f]	302 ± 10[e]	15.1 ± 0.5[g]

Values marked with the same letter are significantly different from each other at p less than 0.05.

1. based on 6-8 observations per group.

W - wheat based diet.
W + L - wheat based diet supplemented with lysine.

or wheat + lysine from weaning onwards for 20 weeks, growth of the lysine deprived group was markedly affected and their growth slowed down at about the same time as the lysine supplemented group.

Table no

Period (wks)	Weight gain (g)				
	Wheat	Wheat + lysine	5%	10%	20%
3 - 11	56	103	21	136	190
3 - 23	126	302	64	300	438
11 - 31 (brought up on specified diet from weaning)	92	68	38	182	44

A comparison of the weight gains of animals in this experiment with those in the previous experiment suggests that the 10% protein group did better than the wheat + lysine group initially (ie during the 3-11 week period) but the body weight gains achieved by the two groups at 23 weeks of age were similar. However, later in life, the 10% protein animals continued to grow steadily even when growth of the 20% protein group slowed down, while the growth of the wheat + lysine group slowed down, as is seen from body weight gains between 11 to 31 weeks of age.

A comparison of the weights gained by groups fed wheat or wheat + lysine for short or prolonged periods, i.e. of animals in expts. 4(a) and 4(b) is presented below.

Age of treatment (weeks)	Weight gain			
	4(a)*		4(b)**	
	Wheat	Wheat + lysine	Wheat	Wheat + lysine
3 - 11	56	103	40	144
13 - 21	47	86	64	110
26 - 34	37	59	10	21

* The diets specified fed for the duration indicated
** The diets specified fed throughout

Weight gained by 4(b) animals are not more than those gained by 4(a) animals in corresponding periodsof time, ruling out the possibility of adaptation to the low lysine diet with prolonged feeding.

At 23 weeks of age half the 4(b) animals fed the lysine deficient diet from meaning were continued on a lysine deprived diet while the other half were switched to a lysine supplemented diet for a period of 12 weeks. During this period no significant differences inthe weight gains of the two groups were found. The group which was fed the lysine supplemented diet from 3-23 weeks of age was also divided into two groups at the age of 23 weeks. One group was continued on the lysine supplemented diet while the other was switched

to a lysine deficient diet. The latter group showed a slightly lower weight at the end of 12 weeks (Fig. 5).

These observations suggest that adaptation to diets low in protein content but good in quality is easier than one which is lacking in critical amino acids. The difference between the long term responses to 5% and 10% casein diets and to wheat diets with and without lysine could be due to the relative lack of methionine in wheat as compared to casein and the greater requirement of older animals for methionine for the growth of fur. Ref 9

The results on biochemical analyses are given in Tables 56 and 57. Blood hemoglobin, serum protein, liver weight and liver protein are reduced in the 3 week group as a result of lysine deprivation. A similar effect, but of a smaller order is seen in the 13 week old group. Liver protein concentration decreased as a result of lysine deprivation in all age groups, though the decrease did not reach significance for the 26 week old group. Animals subjected to lysine deprivation for 20 week in the 2nd part of the experiment also showed significantly lower blood hemoglobin and serum protein. The blood hemoglobin and serum protein of these animals at 23 weeks of age and those of the 13 + 8 week old animals of 4(a) are tabulated below

Age of treatment (weeks)	Expt.	Blood hemoglobin (g/100 ml)		Serum protein (g/100 ml)	
		Wheat	Wheat + lysine	Wheat	Wheat + lysine
3 - 11	4 (a)	10.0	12.7	5.90	6.20
3 - 21	4 (a)	10.3	11.9	6.25	6.51
3 - 23	4 (b)	11.2	12.6	6.28	6.64

These observations on blood hemoglobin and serum protein are consistent with those made on body weight gains and follow the expected pattern of adaptation to the wheat diet with prolonged treatment.

As mentioned earlier counts per minute per g serum protein were determined periodically after injecting 1-C-14-DL leucine, intraperitoneally. The results on loss of label from serum protein are presented in Table 58.

In the 3 week group no significant difference was found in the counts from serum protein of wheat and wheat + lysine fed animals.

In the remaining groups, the serum of the wheat fed group gave more counts than those of the lysine supplemented animals. The differences in counts were significant in the 13 week group between 18-48 hours after injection; in the 26 week

Table 56 : Nutritional status as judged by blood hemoglobin, serum protein, liver weight and liver protein of rats fed a wheat diet with and without addition of lysine.

Age (wks)		Diet	Blood hemoglobin g/dl	Serum protein g/dl	Liver weight (g)	Liver protein g/100g
Initial	Final					
3	11	W	10.0 ± 0.5[a]	5.90 ± 0.07[c]	2.55 ± 0.13[f]	16.1 ± 0.6[g]
		W + L	12.7 ± 0.4[a]	6.20 ± 0.10[c]	3.91 ± 0.18[f]	25.1 ± 0.4[g]
13	21	W	10.3 ± 0.3[b]	6.25 ± 0.05[d]	9.81 ± 0.36	18.6 ± 0.8[h]
		W + L	11.9 ± 0.4[b]	6.51 ± 0.05[d]	8.92 ± 0.57	24.3 ± 0.6[h]
26	34	W	11.6 ± 0.6	6.29 ± 0.09	9.35 ± 0.42	22.8 ± 0.4
		W + L	11.1 ± 0.5	6.46 ± 0.17	8.12 ± 1.08	23.5 ± 0.9
52	60	W	12.4 ± 0.5	6.33 ± 0.15	9.48 ± 0.26	20.3 ± 0.6[i]
		W + L	11.9 ± 0.3	6.60 ± 0.15	9.32 ± 0.34	23.6 ± 1.0[i]
3	23	W	11.2 ± 0.2	6.28 ± 0.12[e]	-	-
		W + L	12.6 ± 0.3	6.64 ± 0.07[e]	-	-

1. based on 6-8 observations per group.
   Values marked with the same letter are significantly different from each other at p less than 0.05.

W - wheat based diet.
W + L - wheat based diet supplemented with lysine.

Table 57 : Values for lysine supplemented group as per cent of wheat group - values for data presented in Tables 52 and 52.

Parameter	Age (wks) during treatment				
	3-11	3-23	13-21	26-34	52-60
	Values for W + L as % those for W				
Body weights at end of treatment	149	205	109	100	100
Weight gain	184	240	183	160	86
Blood hemoglobin	127	113	116	97	86
Serum protein	105	106	104	103	104
Liver weight	153	-	91	87	98
Liver protein	160	-	131	103	116

W - wheat based diet.

W + L - wheat based diet supplemented with lysine.

Table 58 : Incorporation of C^{14}-DL-leucine into serum protein in rats fed on wheat diets with or without addition of lysine at different ages.

Age (wks) at start + period of treatment		Hours after injection						
		12	18	24	48	60	86	105
1	2	3	4	5	6	7	8	0
		cpm per g serum protein - mean ± se[a]						
3 ± 8	W	2351±236	1703±72	1353±232	1115±78	842±63	467±31	371±97
	W + L	2381± 89	1511±52	1272± 58	1030±61	648±49	439±37	338±28
	(W/W+L)100	99	113	106	108	130	106	110
13 ± 8	W	2401± 67	2021±85	1891± 69	1392±70	1187±78	811±58	529±37
	W + L	2118± 71	1480±100**	1364± 33**	1095±26*	1010±29	805±24	691±18
	(W/W+L)100	113	137	139	127	118	101	77
26 ± 8	W	2287± 75	1889±39	1752± 41	1500±51	1451±60	878±45	385±30
	W + L	2348± 74	1860±71	1740± 59	1239±30*	1119±29*	753±28*	562±21
	(W/W+L)100	97	102	101	121	130	117	69

contd...

Table 58 : contd.

1	2	3	4	5	6	7	8	9
52 ± 8	W	2038±893	1768±89	1610±69	1389±29	1140±18	820±38	480±41
	W + L	2061±79	1661±50	1478±39	1241±50*	722±28**	630±28*	348±40*a
	(W/W+L)100	99	106	109	112	158	130	138
3 ±20	W	2102±123	1719±95	1337±67	980±56			
	W + L	2194±13	1726±116	1258±118	884±62			
	(W/W+L)100	96	100	106	111			

W - wheat based diet

W+L - wheat based diet supplemented with lysine.

a. based on 6-8 observations.

One, two and three asterisks respectively indicate significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

group between 48-86 hours after injection; and in the 52 week group between 48-105 hours after injection.

Thus, the loss of labelled luecine from serum protein seemed to be alower in all the wheat fed animals, except in the case of the 3 week group. Also this effect became pronounced with time in the older animals. Significant differences persisted only for a few hours.

Selected data obtained in this experiment and inthe previous one are presented below :

Expt.	Age of treat-ment	Diet	hours after injection			
			12	18	24	48
3	3-11	5% protein	1361	1297	1185	708
3		10% protein	1571	1577	1400	899
3		20% protein	2044	1990	1702	1234
4(a)		wheat	2351	1703	1353	1115
4(a)		wheat + lysine	2381	1511	1272	1030
3	3-20	5% protein	1352		979	
3		10% protein	1655		1422	
3		20% protein	2105		1814	
4(a)	13-21	wheat	2401	2021	1891	1392
4(a)		wheat + lysine	2118	1480	1364	1095
4(b)	3-23	wheat	2102	1719	1337	980
4(b)		wheat + lysine	2194	1726	1258	884

From the above and from Table 58, it can be seen that in the 4(b) groups animals fed wheat or wheat + lysine for prolonged

periods (3-23 wks), no differences existed with regard to the rate of loss of labelled leucine from serum protein. This is in contrast to the adaptation shown by the 4(a) group 13 week old animals subjected to short term lysine deprivation.

Differences in pattern also exist between animals fed wheat and wheat + lysine diets (Expt. 4b) and those fed different levels of protein (Expt. 3) for prolonged periods. The low protein animals seem to adapt to the stress very well unlike the wheat or wheat + lysine fed animals.

This lack of adaptation to the stress is also evident in the wheat and wheat + lysine group animals at 11 weeks of age. This is in contrast to the adaptation seen in the 5% and 10% protein groups.

The counts obtained for the 20 week old 20 % protein group and those for the 21 week old wheat group are comparable at 12 hour while the 24 hour value for the wheat + lysine group is similar to that of the 10% protein group. That this is not due to the fact that in the present study the animals had been on a 20 % protein diet for 13 weeks i.e. before the start of experiment while the animals of experiment 3 had been receiving the various diets from weaning onwards, is evident because the counts obtained in the 23 week old groups fed wheat or wheat + lysine from weaning onwards also show the same pattern.

In summary, the results show that younger animals are more adversely affected by lysine deprivation than the older animals. While no adaptation is seen with regard to growth as a result of prolonged lysine deprivation, a tendency for hemoglobin and serum protein levels to return to normal, in spite of continued stress, is evident. The results on label lost from serum protein show that the older animals adapt better to lysine deprivation by reduced turnover of protein or increased reutilization of amino acids. However, prolonged lysine deprivation impairs subsequent lysine utilization ability of animals.

This observation contrasts with that of Osborne and Mendel (1915) who observed resumption of growth in their animals on feeding of complete protein diets after tryptophan deficient maize diets for prolonged periods. However, adaptive mechanisms for different essential amino acids are different (Chu and Hegsted,1976) so that what may be true for tryptophan may not hold for lysine.

Experiment - 5

Response of bone composition to different amounts of dietary calcium in relation to age at treatment.

The studies described hitherio were concerned with the responses of animals to diets varying in food energy, protein content and protein quality. Another nutrient whose supply varies markedly in different diets and whose utilization is demonstrably modified by the supply in the diet and the requirements of the body is calcium. In fact, mineral homeostasis is governed primarily by the regulation of absorption and excretion whereas that of nutrients providing food energy is governed by the regulation of intake. However, even in the case of minerals, mechanisms may operate to protect the organism from intakes far above the levels at which the body can maintain homeostasis by the regulation of absorption and excretion, a capacity markedly evident in the case of iron and sodium.

Most diets based on cereals contain 400-500 mg of calcium and those based on rice or maize contain even less. On the other hand, there are pastoral tribes consuming about 2 litres or more of milk daily (Leitch, 1961; Gaulin and Konner, 1977). The upper classes in Gujarat may consume as much as 1500-2000 mg as they consume 700-1000 ml of buffalo milk per day which contains more than 200 mg calcium per 100 ml (Rajalakshmi, 1975).

Balance studies with respect to calcium have yielded conflicting results. School boys studied by Nicolls and Nimalasuriya (1939) were found to retain as much as 90% of the calcium ingested whereas an average utilization of 32% is assumed by Mitchell and Curzon (1939) for translating metabolic requirements to nutritional allowances.

The requirements for calcium consist of two components, ~~mineral=accretic~~ namely, that required for mineral accretion in the bone and replacement of loss due to endogenous metabolism. As such, requirements as well as the efficiency with which the mineral is utilized and the capacity of the animal to adapt to marked changes in the calcium content of the diet can all be expected to vary with age.

Another dimension has been added to the problem by the study of Henry and Kon (1947) in which the proneness of rats for osteoporosis in later life was found to be influenced by calcium intakes in early life with a higher prevalence in animals fed high calcium diets during this period.

The present investigations were designed in this context to investigate the following aspects :

(1) response to diets varying in calcium content (100, 440 or 500 mg per 100 g diet) as a function of age.

(2) response to a change in dietary calcium content from low or high levels to the moderate level used in the Hawk-Oser salt mixture (viz. 440 mg per 100 g diet).

The responses were studied in terms of body weights and bone composition in addition to routine estimations of blood hemoglobin and serum protein.

Groups of male rats aged 3, 13, 26 and 52 weeks were fed for 5 weeks, 100, 440 or 600 mg. of calcium per 100 g diet. At this point blood hemoglobin and serum protein were determined and half the animals in each group, matched with the rest for body weight and other parameters, killed for analysis of the femur. The remaining half were continued on a diet providing per 100g, 440 mg Ca for a period of 4 weeks and investigated for the parameters described. The body weights of the various groups initially and at the end of each phase are given in Table 59 and weight gains in Table 60. The low calcium diet resulted in reduced weight gain during phase I in the '3', '13' and '26' week old groups. The high calcium diet affected the '3 week old' group adversely but benefited the '52 week old' group.

Rough estimates of food intake in the various groups were made. All cases, where a detrimental effect on growth rate was observed, were associated with reduced food intake. However, while the reduction in food intake was of the order of 10-15%, the weight gains were lowered to a much greater extent, as is evident from the percentage values given in Table 61.

All animals whose growth suffered due to low calcium diet in phase I, continued to show significant deficits in

Table 59 : Body weight of rats fed different amounts of dietary calcium at different ages

Age at start (weeks	Dietary calcium (mg/100g) during phase											
	I		II		I		II		I		II	
	100		440		440		440		600		440	
	0[d]	1[d]	2[d]		0	1	2		0	1	2	
	mean ±se[c]											
3 a	46±1	126±5			46±2	171±4			45±1	131±3*		
b	49±5	135±9*	210±6*		49±3	181±5	270±5		48±1	146±8*	268±8	
13 a	282±5	322±3*			286±3	363±2			288±3	367±2		
b	286±4	318±4*	393±4*		285±3	359±3	413±3		287±4	365±4	418±4	
26a	384±3	388±3*			387±2	415±2			383±5	411±4		
b	384±3	381±3*	408±2*		386±3	418±3	436±2		385±4	415±2	437±3	
52a	507±5	521±5			496±9	516±8			499±8	525±8		
b	501±7	517±7	535±3		509±6	526±4	541±7		505±5	535±3	549±7	

a = animals cut at the end of phase I (5 weeks).

b = animals cut at the end of the phase II (4 weeks).

c = based on 6 observations

d = 0, 1 and 2 respectively denote initial body weights and body weights at the end of phase I & II.

* = indicate significance at p 0.05. The group receiving the 440 mg Ca. diet is taken as control.

Table 60 : Body weight gains of rats fed different levels of calcium at different ages.

Age at start (weeks)	Dietary calcium (mg/100g) during phase					
	I	II	I	II	I	II
	100	440	440	440	600	440
	mean ± se[1]					
3a	80 ± 5*		125 ± 3		85 ± 3*	
b	86 ± 8*	57 ± 4	132 ± 5	65 ± 3	118 ± 8*	73 ± 4
13a	40 ± 2*		77 ± 4		79 ± 5	
b	33 ± 4	57 ± 4*	75 ± 4	32 ± 3	79 ± 3	41 ± 2
26a	4 ± 3*		28 ± 3		28 ± 2	
b	3 ± 2*	20 ± 3	32 ± 1	17 ± 2	31 ± 3	18 ± 1
52a	14 ± 1		17 ± 2		24 ± 2*	
b	16 ± 1	8 ± 1*	17 ± 3	20 ± 3	30 ± 3*	18 ± 1

a. animals cut at the end of phase I. (5 weeks duration).

b. animals cut at the end of phase II (4 weeks duration).

[1] based on 6 animals per group.

* Indicates significance at $p < 0.05$. The group receiving the 440 mg Ca diet is taken as control.

I and II respectively denote weight gains in phase I and II.

Table 61 : Values for low calcium and high calcium diets as per cent of the group fed 440 mg Ca per 100 g diet for data presented in Tables 59, 60 and. 62 .

Parameter	mg Ca per 100g diet in phase		Age (weeks) at start							
			3		13		26		52	
	I	II	I	II	I	II	I	II	I	II
1	2	3	4	5	6	7	8	9	10	11
			Values as % reference value (440 mg Ca per 100 g diet)							
Body weights	100	440	74	78	89	95	93	94	101	99
	600	440	77	100	99	101	99	100	102	102
Weight gains	100	440	64	88	52	178	14	118	82	141
	600	440	68	112	105	128	100	106	40	176
Blood hemoglobin	100	440	88	95	99	100	102	100	106	98
	600	440	102	101	98	98	103	101	104	97
Serum protein	100	440	97	101	101	101	101	99	102	103
	600	440	101	100	99	100	101	98	101	102
Femur composition										
Wet weight	100	440	85	90	94	98	100	100	102	98
	600	440	103	101	99	100	101	102	101	103

contd...

Table 61 : contd.

1	2	3	4	5	6	7	8	9	10	11
Dry weight	100	440	83	83	91	97	100	100	101	100
	600	440	103	100	98	100	101	101	102	103
Ash weight	100	440	79	85	90	94	99	99	102	100
	600	440	102	102	102	90	101	100	101	103
Calcium	100	440	80	86	90	95	100	100	102	101
	600	440	102	102	98	94	100	100	100	104
Phosphorus	100	440	78	84	92	95	104	98	100	99
	600	440	102	100	99	100	102	99	102	104

Phase - I - 5 weeks duration.
Phase - II - 4 weeks duration.

body weights at the end of phase II, that is even after four weeks on moderate calcium diet.

On the other hand, the '3 week old' group, which had suffered growth retardation due to high calcium diet in phase I, caught up with controls at the end of phase II.

Data obtained on blood hemoglobin and serum protein at the end of phase I and II are presented in Table 62. The low calcium '3 week old' group registered significantly lower blood hemoglobin and serum protein levels compared to controls at the end of phase I. The deficits became insignificant at the end of phase II. This suggests that iron utilization may in some way be affected by dietary calcium levels.

The results on bone composition are presented in Table 63. At the end of phase I, the low calciumffed animals which were '3 weeks and 13 weeks at start showed a significantly lower fresh bone weight, dry bone weight, ash weight and calcium and phosphorus content without significantly altering the Ca : P ratio. When the bone composition was expressed as per cent dry weight, no differences remained in any of these groups as compared to controls, showing the higher moisture content in the bone of these groups. Table 64 shows that the moisture content of the bone decreases with age till adult levels are reached around 31-35 weeks of age. The higher moisture content in the femur of these groups may therefore be taken as an indication of delayed maturation of

Table 62 : Blood hemoglobin and serum protein of rats fed different levels of calcium at different ages.

Age at start (weeks)	Dietary calcium mg/100 g during phase I and II					
	100 - 440		440 - 440		600 - 440	
	I	II	I	II	I	II
1	2	3	4	5	6	7
	mean ± se[a]					
	Blood hemoglobin g/dl					
3	10.8 ± 0.6*	11.9 ± 0.6	12.3 ± 0.4	12.5 ± 0.2	12.5 ± 0.4	12.6 ± 0.4
13	12.0 ± 0.5	12.3 ± 0.4	12.1 ± 0.3	12.3 ± 0.4	11.9 ± 0.3	12.0 ± 0.3
26	12.1 ± 0.5	12.2 ± 0.5	11.9 ± 0.4	12.3 ± 0.4	12.3 ± 0.4	12.4 ± 0.4
52	13.1 ± 0.4	12.8 ± 0.5	12.4 ± 0.5	13.1 ± 0.5	12.9 ± 0.4	12.7 ± 0.4
	Serum protein (g/dl)					
3	6.60 ± 0.02*	6.91 ± 0.08	6.84 ± 0.03	6.87 ± 0.03	6.91 ± 0.03	6.86 ± 0.04
13	7.09 ±0.02	7.03 ± 0.02	7.03 ± 0.03	6.94 ± 0.04	6.93 ± 0.03	6.92 ± 0.02
26	7.04 ± 0.02	7.03 ± 0.02	6.94 ± 0.04	7.08 ± 0.04	7.01 ± 0.02	6.93 ± 0.03
52	7.07 ± 0.04	7.15 ± 0.04	6.93 ± 0.04	6.92 ± 0.04	7.02 ± 0.02	7.04 ± 0.02

a. based on 6 animals per group.

I and II denote phases I (5 weeks) and II (4 weeks).

* Values significantly different at $p < 0.05$ from the group receiving the diet providing 440 mg Ca throughout.

Table 63 : Composition of the femur of rats fed different levels of calcium at different ages.

(A) Effects on absolute composition.

Age at start (weeks)	Dietary calcium mg/100g during phase I and II					
	100 - 440		440 - 440		600 - 440	
	I	II	I	II	I	II
1	2	3	4	5	6	7
	mean ± se[a]					
	Wet weight (mg)					
3	382±6*** (85)[b]	586±6*** (90)	450±9	650±7	462±4	656±9
13	644±5*** (94)	712±4* (96)	688±10	726±4	681±9	728±7
26	818±3	860±10	818±5	863±6	826±5	879±7
52	1098±27	1056±15	1080±20	1078±12	1087±17	1110±25
	Dry weight (mg)					
3	189±4*** (83)	349±5*** (83)	229±5	410±5	237±3	410±5
13	416±4*** (91)	472±3* (97)	456±7	485±3	447±7	484±5

contd...

Table 63 : contd.

1	2	3	4	5	6	7
26	554±2	588±5	555±3	590±5	560±5	597±5
52	754±16	743±7	745±12	745±7	757±15	771±6
			Ash weight (mg)			
3	89±2*** (79)	211±3*** (85)	112±3	247±4	114±1	251±4
13	242±2*** (90)	272±3** (94)	260±4	287±3	266±3	257±7
26	334±2	359±4	337±4	364±6	339±2	364±7
52	475±12	468±9	464±11	469±6	468±9	485±13
			Calcium (mg)			
3	318±0.6*** (80)	74.9±1.3*** (86)	40.1±1.0	87.4±1.7	41.0±0.5	89.0±1.7
13	86.6±0.8 (90)	96.1±1.3 (95)	96.4±1.7	101.6±1.3	94.2±1.1	100±2±2.7
26	118.8±0.9	127.6±1.9	119.2±1.7	128.3±2.6	119.0±1.4	128.3±2.6
52	168.5±4.4	166.4±3.5	164.1±3.5	165.4±1.8	164.4±2.4	171.7±2.4
			Phosphorus (mg)			
3	14.9±0.4*** (78)	36.0±0.6*** (84)	19.1±0.6	42.6±1.1	19.4±0.2	43.0±0.5
13	41.2±0.7** (92)	46.6±0.4 (95)	45.0±0.8	48.8±0.9	44.6±0.6	48.9±1.6

contd...

Table 63 : contd.

1	2	3	4	5	6	7
26	57.6±1.4	59.4±0.8	55.5±0.6	60.8±1.7	56.5±0.5	59.9±1.7
52	78.7±2.7	76.9±1.3	78.5±2.0	77.9±1.1	79.8±2.3	80.6±3.2
			Calcium/phosphorus			
3	2.70±0.09	2.08±0.02	2.10±0.03	2.05±0.04	2.11±0.03	2.07±0.03
13	2.12±0.03	2.06±0.03	2.14±0.04	2.08±0.09	2.11±0.02	2.05±0.03
26	2.13±0.5	2.15±0.04	2.15±0.04	2.12±0.02	2.11±0.04	2.15±0.03
52	2.14±0.02	2.17±0.04	2.09±0.04	2.14±0.03	2.11±0.04	2.14±0.03
B Effect on composition as per cent wet bone.						
			Moisture			
3	50.5±0.5* (104)	40.4±0.5** (109)	48.7±0.4	37.0±0.5	48.8±0.3	37.4±0.3
13	35.5±0.4** (105)	34.1±0.2	53.7±0.3	33.2±0.3	34.4±0.3	33.5±0.3
26	32.3±0.2	31.6±0.4	32.2±0.2	31.7±0.2	32.0±0.2	32.1±0.1
52	30.6±0.4	30.2±0.3	31.0±0.3	30.9±0.3	30.4±0.3	30.6±0.2
			Ash			
3	23.5±0.2** (96)	36.0±0.3** (95)	24.6±0.2	37.9±0.4	24.7±0.4	38.3±0.4
13	37.5±0.2 (96)	38.3±0.2** (97)	39.1±0.4	39.5±0.3	39.2±0.3	39.6±0.3

contd...

Table 63 : contd.

1	2	3	4	5	6	7
26	41.2±0.3	41.7±0.3	41.2±0.3	41.4±0.4	41.0±0.2	41.4±0.5
52	43.1±0.4	44.3±0.2	43.0±0.4	43.5±0.3	42.7±0.2	43.7±0.3
			Calcium			
3	8.3±0.1** (93)	12.8±0.2* (95)	8.9±0.1	13.5±0.2	8.9±0.1	13.6±0.2
13	13.4±0.2* (96)	13.5±0.3 (96)	14.0±0.1	14.0±0.3	13.8±0.1	13.8±0.4
26	14.5±0.2	14.8±0.2	14.6±0.3	14.9±0.4	14.4±0.3	14.6±0.4
52	15.4±0.2	15.8±0.2	15.2±0.2	15.3±0.2	15.1±0.1	15.5±0.2
			Phosphorus			
3	3.9±0.1	6.1±0.1	4.2±0.1	6.6±0.2	4.2±0.1	6.6±0.1
13	6.4±0.1	6.6±0.1	6.5±0.1	6.7±0.2	6.6±0.1	6.7±0.2
26	7.0±0.5	6.9±0.1	6.8±0.2	7.1±0.3	5.9±0.1	6.8±0.2
52	7.2±0.1	7.3±0.1	7.3±0.1	7.2±0.1	7.3±0.1	7.3±0.1
(C) Effect on composition as per cent dry bone.						
			Ash			
3	46.8±0.6	6.05±0.6	48.5±0.6	60.2±0.7	47.7±0.8	61.2±0.8
13	58.1±0.5	57.8±0.5	59.0±0.6	59.1±0.4	59.7±0.6	59.4±0.6

contd...

Table 63 : contd.

1	2	3	4	5	6	7
26	60.3±0.5	61.0±0.4	60.7±0.6	61.7±0.8	60.6±0.5	61.0±0.8
52	61.5±0.9	63.0±0.7	62.3±0.5	63.0±0.7	61.4±0.5	62.9±0.4
			Calcium			
3	16.8±0.3	21.5±0.3	17.5±0.3	21.3±0.3	17.9±0.3	21.7±0.4
13	20.8±0.2	20.3±0.2	21.2±0.2	20.9±0.2	21.1±0.2	20.7±0.5
26	21.5±0.2	21.7±0.2	21.5±0.3	21.8±0.4	21.3±0.3	21.5±0.3
52	22.4±0.3	22.4±0.3	22.0±0.1	22.2±0.3	21.7±0.2	22.3±0.2
			Phosphorus			
3	7.9±0.2	10.3±0.2	8.4±0.2	10.4±0.1	8.2±0.1	10.5±0.2
13	9.9±0.2	9.9±0.1	9.9±0.2	10.1±0.2	10.0±0.1	10.1±0.7
26	10.1±0.2	10.1±0.1	10.0±0.2	10.3± 0.2	10.1±0.1	10.0±0.7
52	10.4±0.4	10.4±0.4	10.5±0.2	10.5±0.2	10.3±0.2	10.5±0.2
(D) Effect on composition as per cent ash						
			Calcium			
3	36.0±0.9	35.5±0.2	36.2±0.1	35.4±0.2	36.1±0.4	35.5±0.2
13	35.8±0.1	35.2±0.2	35.9±0.3	35.4±0.2	35.3±0.2	35.5±0.2
26	35.6±0.2	35.6±0.2	35.4±0.2	35.3±0.3	35.1±0.3	35.2±0.2
52	35.5±0.2	35.6±0.2	35.4±0.3	35.3±0.3	35.4±0.2	35.4±0.2

contd...

Table 63 : contd.

1	2	3	4	5	6	7
			Phosphorus			
3	16.8±0.4	17.1±0.2	17.2±0.2	17.3±0.3	17.1±0.2	17.1±0.2
13	16.9±0.3	17.1±0.2	16.8±0.3	16.9±0.4	16.7±0.1	17.3±0.3
26	16.8±0.3	16.6±0.2	16.5±0.3	16.7±0.3	16.7±0.2	16.4±0.2
52	16.6±0.2	16.5±0.3	16.9±0.3	16.6±0.2	16.8±0.2	16.6±0.3

I and II denote phases I (5 weeks)and II (4 weeks).

a. based on 6 observations per group.

One, two and three asterisks respectively indicate significance at p less than 0.05, 0.01 and 0.001 from the group receiving the diet providing 440 mg Ca throughout (control)

[b]Figures in parentheses indicate values as percent controls.

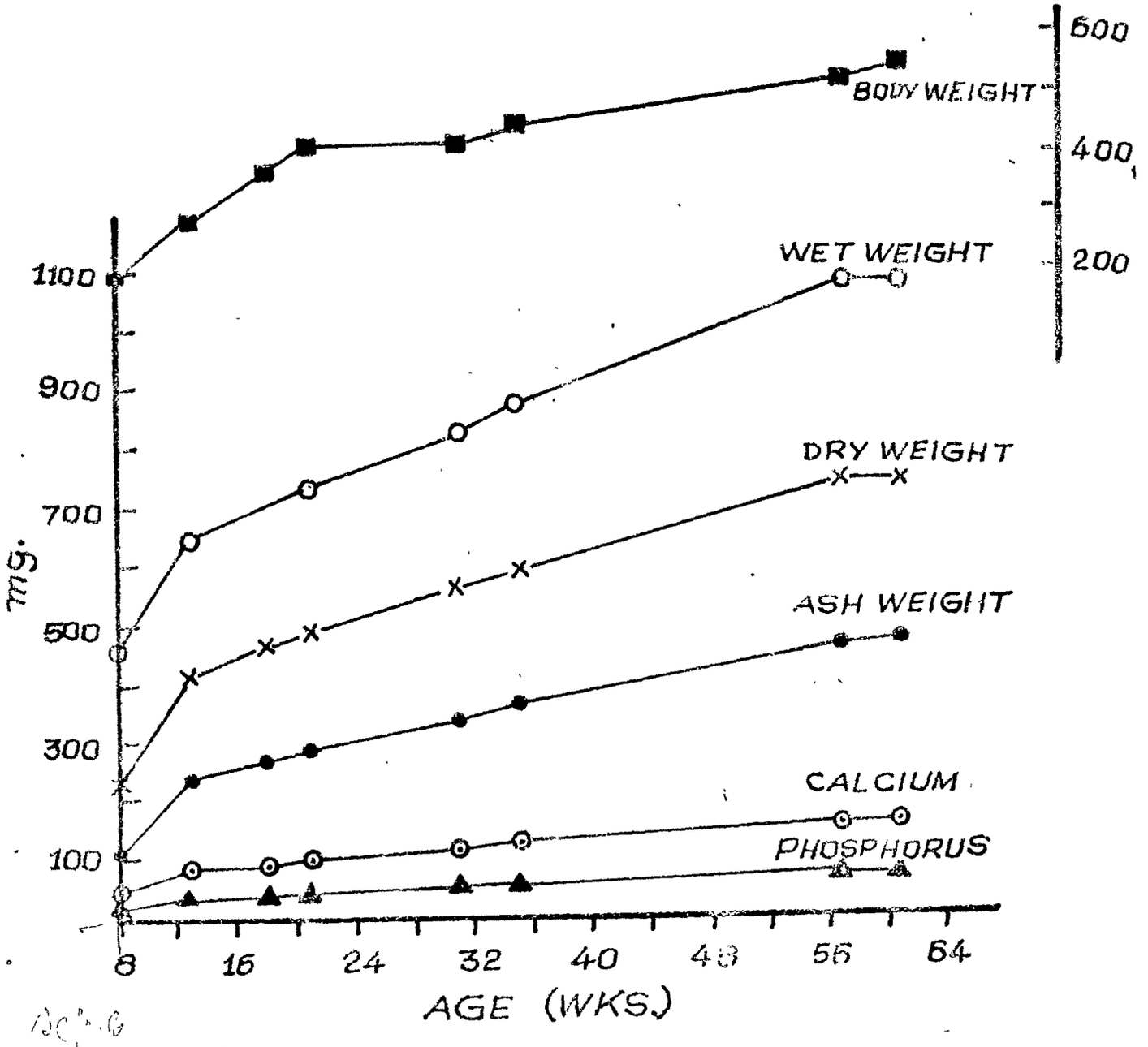
Table 64 : Composition of femur at different ages in rats fed a standard diet providing 440 mg Ca throughout.

g per 100g fresh bone	age in weeks				
	8	13	18-21	31-35	57-61
	mean ± se				
Moisture	49.1 ± 0.6	37.0 ± 0.5	33.5 ± 0.2	32.5 ± 0.1	31.0 ± 0.2
Ash	24.6 ± 0.2	37.9 ± 0.4	39.3 ± 0.3	41.6 ± 0.2	43.2 ± 0.3
Calcium	8.9 ± 0.1	13.5 ± 0.2	14.0 ± 0.2	14.8 ± 0.2	15.2 ± 0.1
Phosphorus	4.2 ± 0.1	6.6 ± 0.2	6.6 ± 0.1	7.0 ± 0.2	7.3 ± 0.1
Ca : P ratio	2.10 ±0.03	2.05 ± 0,04	2.11 ± 0.02	2.14 ± 0.02	2.12 ± 0.01

a. values based on 6 animals per group

b. Absolute values for body weight and bone composition are presented in Fig. (6).

FIG.-6-EFFECT OF AGE ON COMPOSITION OF FEMUR IN RATS FED A STANDARD DIET PROVIDING 440 mg Ca THROUGHOUT.



the bone. This has been reported in earlier studies in this laboratory (Dave, 1977).

The low calcium diet was found not to have any effect on bone composition at the end of phase I in animals which were 26 weeks and 52 weeks at start. The high calcium diet did not affect the bone composition in any of the groups studied.

At the end of phase II, the femur composition of the low calcium fed animals, which were 3 weeks and 13 weeks at start, remained different from that of the controls. However, some tendency for recovery was evident, considerably more in the '13 week old' group than in the '3 week old' group.

The calcium content of the low and high calcium diets was respectively 23% and 136% of the calcium content of the moderate calcium diet. However, the calcium in the bone at the end of phase I was 85% and 103% for the '3 week old' groups and 94% and 99% for the '13 week old' groups. At the end of phase II the values were 90% and 103% for the '3 week old' group and 98% and 100% for the '13 week old' group.

These observations indicated the operation of a regulatory mechanism to preserve the bone composition as near normal as possible.

A delayed effect of the low calcium diet during phase I was observed in the case of the '52 week old' group.

The weight gain during phase II in this case was significantly less than in animals of the same age which were fed moderate calcium in phase I. A similar picture was obtained with regard to calcium increment in the femur during phase II. Calcium increment during phase II, in the case of the '52 week old' group fed the high calcium diet in phase I, was considerably more than in animals of the same age which were fed the moderate calcium diet during phase I. The above observations on calcium increment during phase II emerge from the table below:

Age at start Weeks	Ca in diet (mg per 100g)		
	100	440	600
	Increment in Ca of femur during phase II		
3	43.1	47.3	48.0
13	9.5	6.2	6.0
26	8.8	9.1	9.3
52	-2.1	1.3	7.3

The results of the experiment are summarized below:

(1) Feeding a low calcium diet resulted in significantly lower body weights and body weight gains in all but the 52 week old group. The high calcium diet had an adverse effect on body weight gains in the 3 week old group and a beneficial effect in the '52 week old group

indicating a requirement of more than 440 mg/100 g diet in old age.

(2) Blood hemoglobin and serum protein resulted in a significant fall in the '3 week old' group only. This suggested some interaction between iron and calcium metabolism, dependent on age. The levels became normal on feeding of a moderate calcium diet for 4 weeks.

(3) The low calcium diet resulted in altered bone composition in the younger groups. The wet weight, dry weight, ash weight, calcium and phosphorus content decreased and moisture increased, without altering significantly the composition of the dry bone and ash. This indicates delayed maturation of the bone in the young, low calcium fed animal. The high calcium diet had no effect on bone composition in any of the groups.

(4) The deficits found with regard to bone composition in the younger low calcium fed groups at the end of phase I persisted at the end of phase II although some catchup was evident, considerably more in the '13 week old' group than in the '3 week old' group.

(5) Compared to controls during phase II increments in bone constituents were more in the high calcium fed '52 week old' group, but less in the corresponding low Ca animals, indicating delayed effects of previous dietary calcium intake.

This experiment showed an adverse effect of low calcium diets on bone weight and composition at an early age in rats. In contrast, whereas the recommended allowance of calcium is 500-600 mg/day (Gopalan and Narasingarao, 1971), satisfactory skeletal development of children in calcium intakes as low as 200 mg/day has been reported by investigators in Ceylon, South Africa and India. (Nicolls and Nimalasuriya, 1939; Walker, 1958, 1961). A reason for the difference may be an increase in calcium requirements on high protein diets as suggested in several studies (Johnson et al, 1970; Anand and Linkswiler, 1974; Linkswiler et al, 1971). In humans, low calcium diets arise essentially due to an overall nutritional deficiency, whereas in rats, the experiments are done on rats fed low calcium but high protein diets.

Henry and Kon (1947) studied the effect of calcium deficiency and calcium excess on calcium retention at different ages. He found that calcium retention decreased as the age of the rat increased. This effect was least in the calcium deficient group, suggesting that calcium

retaining ability was not permanently impaired in the calcium deprived group. These observations lend support to the observations made in the present study. Studies conducted over long periods (Sherman and Boocher, 1931; Henry and Kon, 1953) have shown that animals on low calcium intakes deposit calcium slowly but steaily and do not register any permanent adverse effects on the skeleton. On the basis of these studies, one would expect quick catch-up on rehabilitation. This was not found to be the case in the present studies, although tendency for catch-up was evident, considerably more in the '13 week old' group than in the '3 week old' group. It has been shown that absorption and utilization of calcium is influenced by the overall nutritional status of the animal (Wasserman, 1963). The poor nutritional status of the 3 week group as judged by its low blood hemoglobin at the end of phase I, may be responsible for impairing absorption and utilization of available calcium on rehabilitation. It is possible that the period of moderate calcium feeding was too short to reverse the effects of deficiency.

In conclusion, the response of the animal to very low or high calcium diets is age dependent. There seems to be a regulatory mechanism to preserve bone composition as near normal as possible. The recovery from adverse effects of low dietary calcium on feeding of a moderate calcium diet may be dependent on age as well as nutritional status of the animal.

Experiment - 6

The utilization of carotene in rats depleted of vitamin A in relation to dietary vitamin A source (vitamin A or carotene) prior to depletion.

It will be evident from the foregoing that the efficiency with which nutrients such as food energy, protein and calcium are utilized is influenced by the amounts suppliedin the diet as well as the age and previous dietary history of the animal.

In diets based on plant foods, carotenoids, particularly, β-carotene, serve as the precursor for vitamin A. Thus the utilisation of this nutrient depends not only on the amount supplied but also the efficiency with which it is converted to vitamin A. This efficiency appears to depend onthe source of β-carotene,(Kemmerer and Fraps, 1938, 1945; MRC, 1949) the amount in which it is consumed and the age of the animal. Marked species variations are also found (See Table 6).

The studies on the utilization of carotene and vitamin A requirements in man have been reviewed by Moore (1957), Mitchell (1964), NAS-NRC (1962) the (Mantisch and Bauernfield, 1963 Ullrey,1972; Rajalakshmi et al, 1975). The same suggest a very wide variation in the values arrived. This could be due to the factors mentioned above, but one possibility that has been ignored in considering these studies is the dietary history of the individual or the group with special reference

to the amounts of vitamin A derived in the form of B-carotene in their habitual diets. That this might be a significant variable for other nutrients such as calcium is suggested by variations in the ability of individuals to utilize calcium from phytate-rich diets. This problem was sought to be investigated by studying the utilization of β-carotene in animals fed on either vitamin A or carotene as the only source of this vitamin in the immediate postweaning period and then deprived of either till the liver stores were believed to be exhausted and then repleting them with β-carotene.

Weanling male rats were used in the study. Two groups of five animals each matched for initial body weight were fed a diet supplemented with either 50 µg of vitamin A per 100 g or 150 µg β-carotene per 100 g. In previous studies these levels were found to be quite adequate to maintain normal liver stores of vitamin A which were also found to be comparable in the two groups. At the end of 8 weeks both the groups were fed a diet free of vitamin A as well as carotene till the growth of the animal stopped.

In previous studies in this laboratory, the onset of growth arrest was found to be associated with the disappearance of vitamin A from the liver (Chari, 1967). A similar conclusion can be drawn from the studies reviewed by Mitchell (1964).

At this stage serum vitamin A was measured in both groups and all the animals switched to a diet providing only β-carotene as vitamin A source at the level specified. Body weights were monitored throughout and serum vitamin A assayed again at the end of the treatment period.

Data presented in Fig. 7 and Table 65 show the growth rate in the two groups to be comparable. This was not unexpected. Incidentally, while growth arrest is found with total depletion and the minimum amount required for growth resumption has been used as standard, growth is not linearly related to vitamin A supply in the diet.

When subjected to the depletion diet, the two groups showed virtual growth ~~stress~~ arrest at about the same time. This confirms our previous impressions of the approximate equivalence of 3 µg of β-carotene and 1 µg of vitamin A.

The serum vitamin A levels were comparable in both groups at this point and corresponded to the level (13-15 µg per 100ml found in previous studies in this laboratory (Rajalakshmi et al, 1975) in animals with no measurable amount of vitamin A in the liver. It is well known that even after depletion of liver stores, minimal levels of serum vitamin A are maintained for a fairly long time, presumably from small amounts derived from other tissues such as the kidney or present inthe liver in trace amounts.

FIG.-7-GROWTH OF RATS FED VITAMIN A OR CAROTENE IN EARLY LIFE & REPLETED (23-26 WKS) WITH CAROTENE AFTER AN INTERVENING PERIOD OF DEPLETION (11-23 WKS).

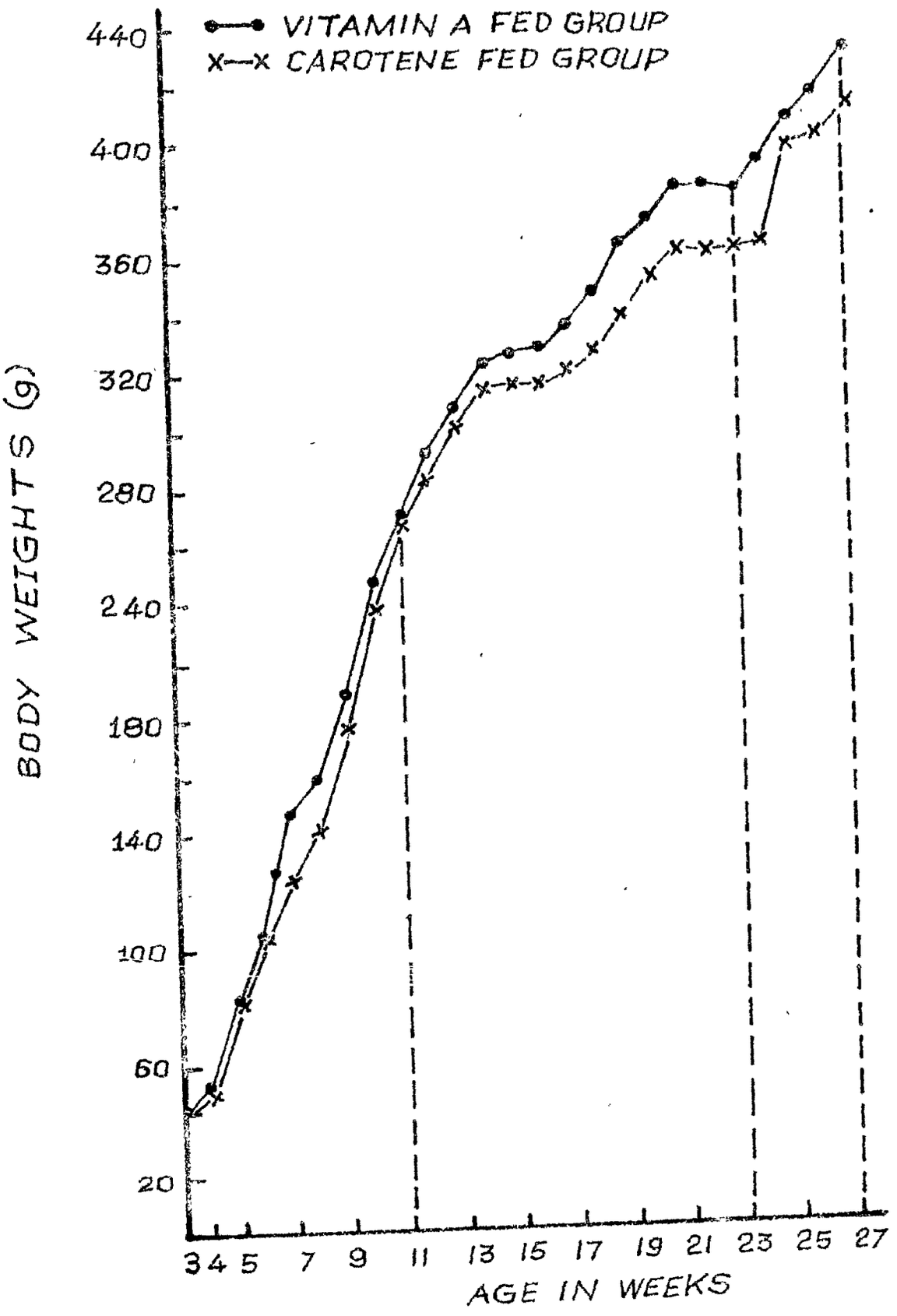


Table 65 : Body weight gain during periods of depletion and repletion in rats fed vitamin A in carotene prior to depletion.

Group and animal no.	Initial body wt. (g)	Body weight gain(g) during weeks								
		3-11	11-20	20-21	21-22	22-23	23-24	24-25	25-26	26-27
Previously vit.A fed										
1	44	315	89	6	0	1	10	18	-8	29
2	44	199	99	16	0	2	2	8	-33	15
3	40	198	115	20	-1	-1	9	32	+12	8
4	44	180	85	20	-1	-3	10	7	4	11
5	44	214	105	-1	-4	-4	24	11	+8	23
mean ± se	43 ± 1	201±7	99±6	12±5	-1±1	-1±1	11±4	15±5	-3±9	17± 4
Previously carotene fed										
6	40	214	72	15	+2	-16	6	32	+3	22
7	44	201	86	6	+3	- 1	2	19	+5	18
8	44	171	105	15	-3	+1	13	39	+2	10
9	43	204	74	18	-6	+2	3	48	+2	6
10	45	195	85	1	-0	+2	2	30	+1	8
mean ± se	43±1	198±7	84±7	11±4	1±2	1±1	5±2	34±5	3±1	13±3

The response to repletion with vitamin A was good in both groups (Table 65) but an analysis of the data reveals the differences in weight gain as well as the increments in serum vitamin A. This becomes all the more clear when a scatter gram is plotted for increments in serum vitamin A against increments in body weight for the two groups (Fig. 8) or when both sets of data are arranged in descending order of magnitude.

Vitamin A fed group		Carotene fed group	
Increment in		Increment in	
Body weight	Serum vitamin A	Body weight	Serum vitamin A
52	9.2	57	11.2
42	7.4	56	10.2
39	3.9	51	8.7
22	3.7	42	8.1
-10	2.5	42	7.3

A similar analysis of the data either for the initial period or the depletion period does not show a similar pattern if anything, the previously vitamin A fed animals showed some what greater gains in body weight during the depletion period and had slightly higher levels of serum vitamin A at the end of the depletion period. Thus the better response of the previously

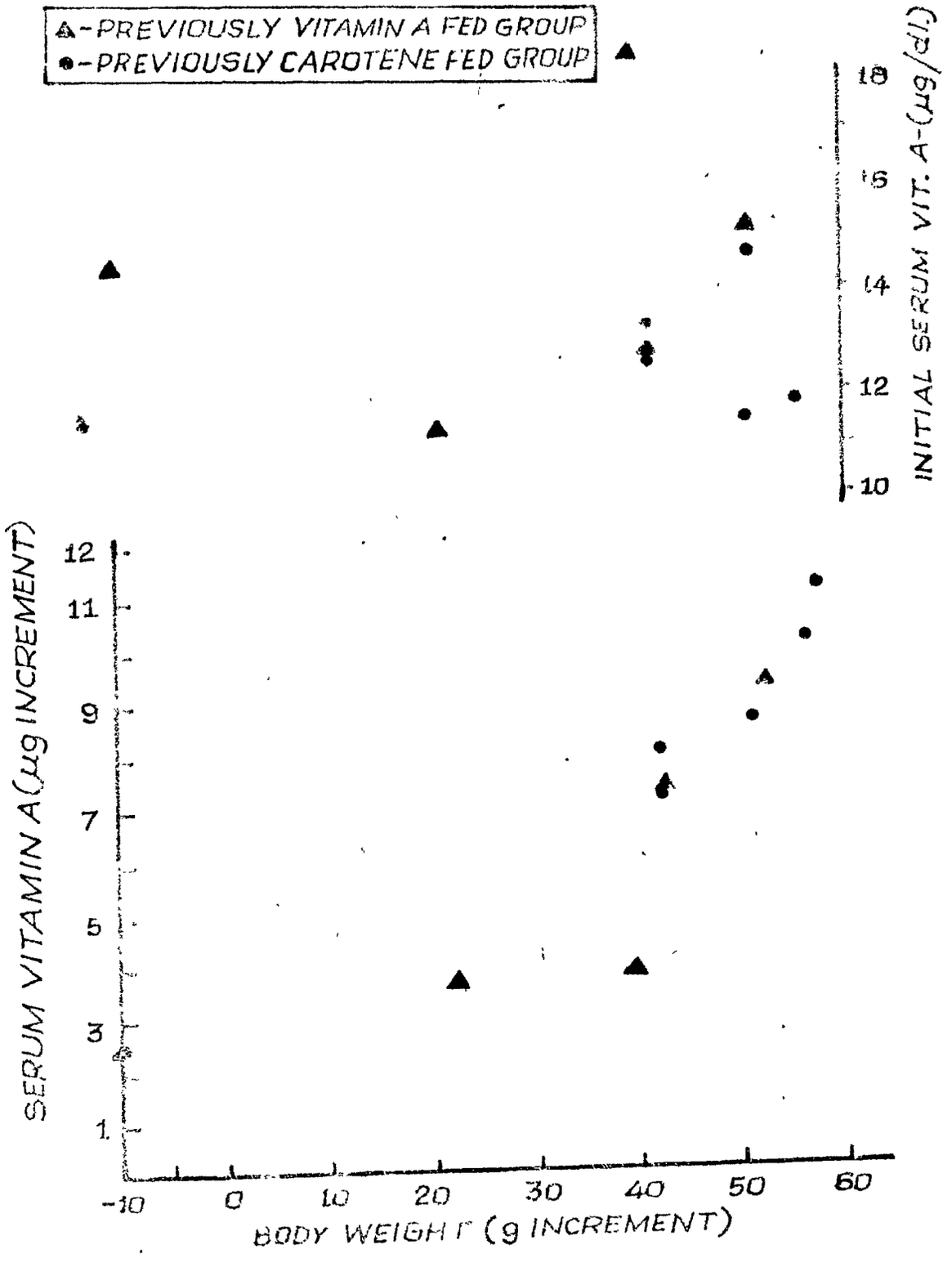
Table 65 : Serum vitamin A during periods of depletion and repletion in rats fed vitamin A or carotene prior to depletion.

Group and animal no.	Serum vitamin A at the age of weeks		increase in serum vit.A between 23 and 26 weeks of age
	23	26	
Previously vitamin A fed			
1	14.3	16.8	2.5
2	15.1	24.3	9.2
3	12.7	20.1	7.4
4	11.3	15.0	3.7
5	18.4	22.3	3.9
mean ± se	14.4 ± 1.4	19.7 ± 1.9	5.3 ± 1.4
Previously carotene fed			
6	12.5	19.8	7.3
7	11.4	20.1	8.7
8	13.2	21.3	8.1
9	14.6	25.8	11.2
10	11.8	22.0	10.2
mean ± se	12.7 ± 0.6	21.8 ± 1.0	9.1 ± 0.6*

* Previously carotene fed group significantly different from previously vitamin A fed group at $p < 0.05$.

FIG.–8.

SCATTER GRAM FOR INCREMENTS IN SERUM VITAMIN A AS RELATED TO INCREMENTS IN BODY WEIGHT FOR RATS FED VITAMIN A OR CAROTENE PRIOR TO DEPLETION.



carotene fed group cannot be attributed to any differences in their favour to begin with.

These results suggest that the efficiency of conversion of carotene to vitamin A is influenced by the previous dietary history of the animal, in particular, whether the vitamin A requirements of the animal have been met by preformed vitamin A or from carotene in early life.

The livers of all the animals were analysed at the end of the experiment to estimate vitamin A stores. No measurable amount of vitamin A was found in any of the animals. The absence of liver stores at this point reinforces the assumption that at the end of the depletion period, livers had been completely deplted. Other investigators have also observed that the serum response to vitamin A in depleted animals precedes the liver response (High, 1954; Dowling and Walker, 1958). Moore (1957) has calculated that 9 µg retinol per day is required in the rat for rebuilding liver stores of vitamin A in depleted rats.

Assuming a conversion ratio of 3:1, 27 µg of carotene a day would be required. The food intake during the period of repletion was 15-20 g an amount which gave22.5-30 µg carotene a day, Perhaps this amount was not sufficient to build up liver stores within the period studied, namely, 3 weeks.

In previous studies (Chari et al 1967), 75 µg of carotene per day was sufficient to build up liver stores in previously depleted animals in a period of 6 weeks. The liver vitamin A in these animals was of the order of 63 µg. It is possible that the liver is replenished, only after the other tissues in critical need of vitamin A are replenished.

In conclusion, the results of this experiment provide evidence that carotene utilization is more efficient in depleted animals fed previously on carotene. Further studies using graded amounts of carotene for repletion, monitoring of the body weights and serum vitamin A levels more frequently in the initial stages of repletion and of liver vitamin A at different points of repletion are required.